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RAPID COMMUNICATION / COMMUNICATION RAPIDE

Results of pop-up satellite tagging of spawning size class fish in the Gulf of Maine: do North Atlantic bluefin tuna spawn in the mid-Atlantic?

Molly E. Lutcavage, Richard W. Brill, Gregory B. Skomal, Bradford C. Chase, and Paul W. Howey

Abstract: Twenty giant bluefin tuna were captured in September and October 1997 and tagged and released with pop-up satellite tags programmed to jettison from March through July, 1998. Seventeen tags successfully released from the fish (12 during the known May–July spawning period), all of which were located north of 33°N latitude, in a region of the mid-Atlantic bounded by Bermuda and the Azores. Our results argue for reconsideration of current assumptions about North Atlantic bluefin tuna migration patterns, mixing rates, spawning areas, and stock structure and highlight the need for additional studies.

Résumé : En septembre et octobre 1997, on a capturé vingt thons rouges géants, que l'on a relâchés après leur avoir fixé des étiquettes détachables pistées par satellite qui devaient se libérer de mars à juillet 1998. Dix-sept étiquettes ont bien été libérées (dont 12 pendant la période de fraye connue de mai à juillet), toutes au nord de 33N, dans une région du centre de l'Atlantique délimitée par les Bermudes et les Açores. Nos résultats portent à reconsidérer les hypothèses actuelles sur les régimes migratoires du thon rouge dans l'Atlantique Nord, le taux de mélange, les zones de fraye, et la structure du stock, et mettent en évidence la nécessité de nouvelles études.

[Traduit par la Rédaction]

The North Atlantic bluefin tuna, *Thunnus thynnus*, is a highly migratory species present in large schooling assemblages in New England and Canadian waters from June through November. Bluefin tuna populations have been reduced by intense fishing pressure worldwide, and U.S. and Canadian fisheries are subject to strict quotas. Since 1982, North Atlantic bluefin tuna have been managed under the International Commission for the Conservation of Atlantic Tunas (ICCAT) as separate east and west Atlantic stocks with substantially different fishing control levels. Mixing rates are estimated at 2% to 7% yearly (Restrepo 1996) but are ignored in stock assessments. However, the status and stock structure of the population remain controversial, and current models of long-term migrations include assumptions on trans-Atlantic movements and spawning site fidelity that have not been extensively tested.

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The majority of bluefin tuna caught in New England are in size classes representative of sexually mature fish (Mather et al. 1995). After foraging in the Gulf of Maine and adjacent continental shelf, bluefin tuna are presumed to disperse to the south and east and eventually to return and spawn in the Gulf of Mexico by April–June (Richards 1976; Rivas 1978; McGowan and Richards 1989). However, during presumed spawning periods, adult bluefin tagged in New England have been recaptured throughout the Atlantic and Mediterranean Sea (Mather et al. 1995), and Japanese longline records also show their continuous distribution across the Atlantic (Shingu and Hisada 1977).

Utilizing a new technology, we undertook a bluefin tagging experiment to identify long-range migration patterns of the New England assemblage. We employed the pop-up satellite tag (model PTT 100 PU1) designed and built by Telemetry 2000, Inc. (Columbia, MD), that was recently proven in short-term deployments (Block et al. 1998). Tags consist of a streamlined carbon–epoxy pressure housing plus antenna, a corrosive release mechanism, and a flotation collar that allows the antenna to float upright when the tag jettisons from the fish. A microprocessor records up to 61 average daily water temperatures (sampled hourly). The re-

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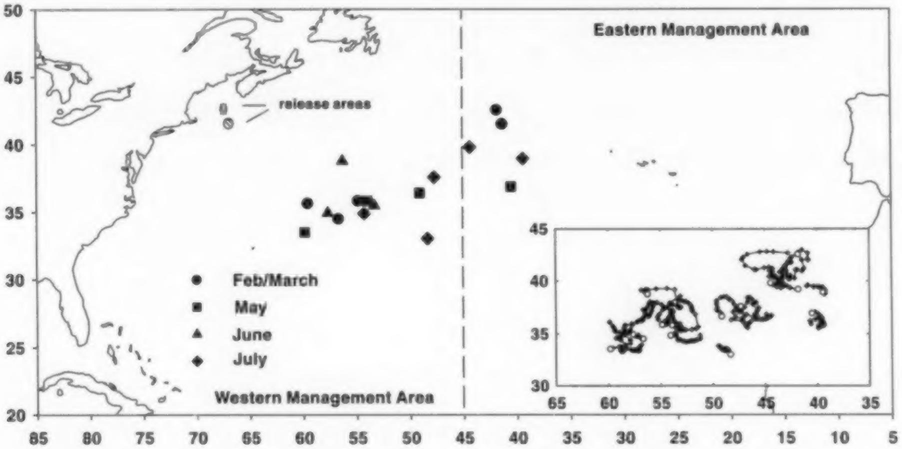
Fig. 1. Jettison locations for pop-up satellite tags deployed on giant bluefin tuna in the Gulf of Maine, 24 September – 6 October, 1997. Insert shows pop-up satellite tag drift patterns after they are released from giant bluefin tuna. Solid circles indicate jettison location and cross hatches indicate daily reporting positions. Scale: 1 deg latitude = 1.85 km.



corded temperature data, along with real-time sea surface temperature, are transmitted for approximately 30 days following jettison. Argos satellites determine the location of the tag and retransmit the data to ground receiving stations. The first 60 average daily water temperature records are obtained during the period immediately following the fish's release. The 61st data record is the average daily water temperature for the day before jettison. Mean values are reported with standard deviations.

Tags were attached to a medical-grade nylon dart using monofilament fishing line. An identification tag was incorporated into the attachment system to allow for collection of a second data record if the fish is recaptured. Twenty giant bluefin tuna were captured (17 by rod and reel, 3 by purse seine), tagged in the dorsal musculature with custom-built tagging applicators alongside the tagging vessels, and released off central New England from 24 September through 6 October, 1997. The total straight fork lengths of four fish (estimated independently by the captain and mate) were 190–200 cm, eight fish were 201–225 cm, and eight fish were 226–263 cm.[3] Tags were programmed to release monthly in groups of five, from late February through July, bracketing the assumed spawning periods in the Gulf of Mexico and Mediterranean Sea. Bluefin reporting locations were plotted on sea surface temperature (SST) fields derived from 9 km resolution Advanced High Resolution Radiometer (AVHRR) NOAA Pathfinder data that were custom processed for 1985–1995 (Arthur Mariano, Rosenstiel School of Marine and Atmospheric Science, University of Miami, Miami, FL 33149, U.S.A., personal communication).

Seventeen tags (85%) successfully released from the fish, and all reported high quality locations and the complete 61 average daily temperature records. At the time of jettison, tagged giant bluefin were located north of 33°N, in the southern temperate and northern subtropical oceanic biogeographic areas along the Gulf Stream and Gulf Stream recirculation region, bounded on the south by the north Sargasso Sea, the Bermuda islands on the west, and the Azores on the east (Fig. 1). Five of seventeen fish were located east of the 45°W ICCAT management line that separates the North Atlantic stock. A continuous eastward travel with time is not apparent, as individuals were present in the easternmost areas in early March as well as in late May and July. There is no apparent trend in distribution by size, as the smallest and largest fish were located along a broad region of the mid-Atlantic. The positions of three individuals that were members of the same school were several hundred miles apart when the tags reported in late February – March.

Because this is the first time that these externally attached tags had been used on long-term deployments, we established that the satellite tags were not jettisoned prematurely by comparing temperature records reported by the tag before and after presumed jettison. The mean coefficient of variation for temperatures before presumed jettison (0.48) was fourfold higher than that occurring after the tags jettisoned (0.14). The drift patterns of tag positions plotted for 30 days after releasing from the fish were primarily circular (Fig. 1 inset). If tags had come off prematurely in the Gulf of Maine, these drift patterns could not reasonably account for their subsequent mid-Atlantic reporting positions.

Sea surface temperatures when the tags jettisoned in late February – March and May–July were 16–19°C and 20–28°C, respectively, and are consistent with climatology records and real-time SSTs from AVHRR archives. The range of mean daily temperatures was 11–27°C (x = 20.4°C ± 5.79). Based on temperature records (Fig. 2), two individuals probably remained in the New England continental shelf area (<16°C) for at least 10 days and one possibly as long as 59 days after tagging, while fourteen apparently traveled to warm Gulf Stream waters within that period. On the day before jettison, mean temperatures for May (20.8 ± 0.61°C), June (23.3 ± 0.80°C), and July (25.6 ± 1.22°C) were considerably warmer

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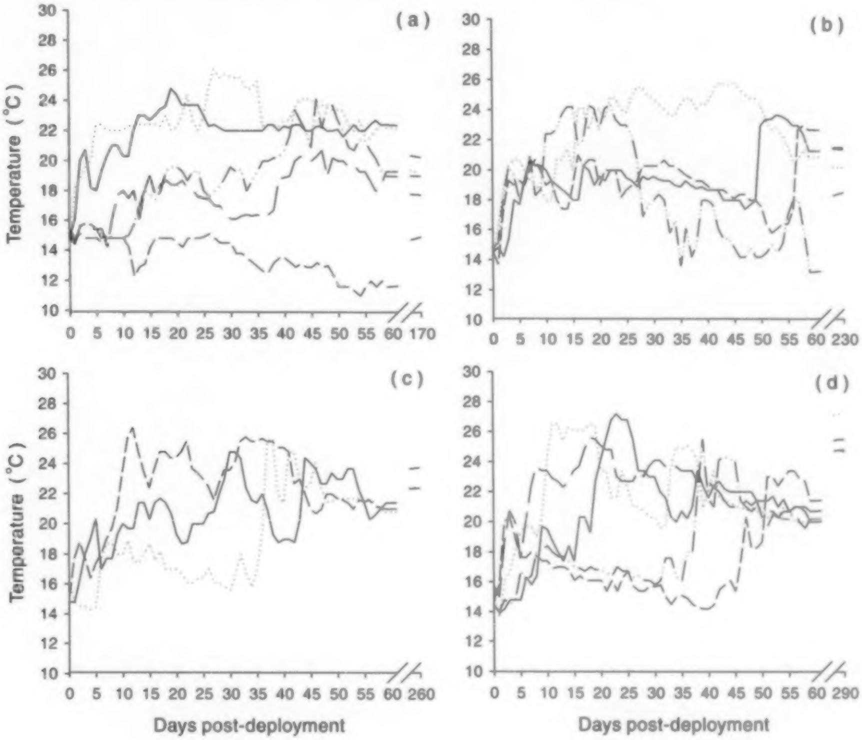
[3] Current bluefin tuna lengths at age estimates (SFL): age 7, 177 cm; age 8, 193 cm; age 9, 207 cm; age 10, 220 cm; age 11, 233 cm; age 12, 244 cm; age 13, 254 cm.

Fig. 2. Mean daily temperatures recorded by pop-up satellite tags deployed on giant bluefin tuna and jettisoned over the following periods: (*a*) 23 February – 5 March, 1998; (*b*) 18–26 May, 1998; (*c*) 19–22 June, 1998; and (*d*) 19–23 July, 1998. The first 60 values are for the 60 days immediately following tagging. Data collection is suspended until the day before jettison, when the tag records the 61st (final) data record.



than the February–March records (17.5 ± 1.07°C). Temperature records from giant bluefin tuna in this study and smaller bluefin tagged in Cape Hatteras (Block et al. 1998) indicate that bluefin experience rapid changes in mean daily temperatures. These gradients could be accounted for by shifts in depth of occurrence and also by travel across fronts at boundary current margins.

The view of bluefin migration indicated by our results differs from the management paradigm that western Atlantic spawning size class bluefin are primarily found in the Gulf of Mexico in late April – June. There are historical accounts of bluefin tuna spawning in the Azores and Canary Islands (Mather et al. 1995), but larvae have not been found there. However, bluefin tuna were consistently documented in the middle and northwest Atlantic in Japanese longline fisheries (Shingu and Hisada 1977) and in the U.S. Bureau of Commercial Fisheries exploratory R/V *Delaware* and *Crawford* cruises in the 1950s–1960s (Mather and Bartlett 1962; Squire 1963). Medium- and giant-sized fish in spawning, near spawning, or recently spent condition were sampled in late spring along the northern boundary of the Gulf Stream off New England (Baglin 1976; Mather et al. 1995). Surprisingly, these observations were not addressed in later studies, perhaps because minimum age of spawning for "western" Atlantic fish remains controversial, and lacking additional confirmation, these views were held in question.

The current paradigm of bluefin reproduction is that spawning occurs in restricted water masses on continental shelves (e.g., edges of the Gulf of Mexico's Loop Current or the Mediterranean Sea), which supposedly support favorable larval tuna habitat (Bakun 1996). Yet strong thermal boundaries, differential advection, and vertical mixing processes are also characteristic of oceanic regions bordered by boundary currents (Olson et al. 1994), and other tunas (e.g., albacore, skipjack, yellowfin) utilize oceanic frontal regions for reproduction (Bakun 1996). Historically, sampling for bluefin tuna has occurred primarily on the continental shelf and not in oceanic regions. In 1985, larval bluefin were found in Gulf Stream water in the South Atlantic Bight, but McGowan and Richards (1989) concluded that the Gulf Stream waters were inappropriate for optimal larval development. Questions then remain as to the disposition of giant bluefin tuna in the mid-Atlantic during the presumed spawning period.

Although we cannot say with certainty that the fish that we tagged were spawning in the mid-Atlantic region, it is a distinct possibility. Most of the tagged fish were within currently accepted spawning sizes, and hydrographic conditions of the summer locations are within SSTs (24–27°C, Richards 1976), salinity, and productivity ranges of Gulf of Mexico spawning grounds. Although entirely speculative at this point, the presence of a mid-Atlantic spawning ground could account for inconsistencies in recent observations regarding the Atlantic bluefin tuna's population biology and migration habits, namely the apparent increase in juvenile and adult age classes on the NW Atlantic fishing grounds (ICCAT, unpublished data, Standing Committee on Research and Statistics, Genoa, Italy, 1998), the decline in numbers of spawners in the Gulf of Mexico, and the consistent failure to find any substantial assemblage of young of the year (age 0s) on the continental shelf. The alternatives that would discount a mid-Atlantic spawning zone include the following: that giant bluefin tuna do not spawn each year, that tagging alters migration behavior, or that all of the tagged fish spawn elsewhere in April or August. Most of these explanations seem unlikely and lack parsimony. Fecundity of bluefin tuna is believed to increase with size (Baglin 1976), and at least six of our tag returns were from individuals estimated to be at least 220 cm SFL. Even if bluefin tuna were to skip a reproductive year, which is not known in the tunas, it is hard to imagine a case where all of the tagged fish (encompassing different age classes) would be on the same reproductive schedule such that none returned to the presumed Gulf of Mexico or Mediterranean spawning areas. Moreover, it seems equally unlikely that disturbance from tagging causes all of the fish to undertake "abnormal" migrations but that all were in the same general direction. Smaller bluefin tuna tagged off Cape Hatteras and at large for up to 90 days had a similar latitudinal distribution along the Gulf Stream margin (Block et al. 1998). An alternative hypothesis that all of the fish spawned in the Gulf of Mexico either in April or August, periods not covered by our tagging results, is unlikely because peak spawning activity and gonadal-somatic index is greatest there in May and June (Richards 1976; Baglin 1976).

The consistency of the locations of tagged giant bluefin tuna during their presumed summer spawning period argues at the very least for reconsideration of current assumptions about migrations, spawning habitat, and stock structure. In a recent analysis of ICCAT tagging data for 1970–1994, only 1% (26 of 21 208) of bluefin tuna released off North America were taken in the eastern Atlantic (Restrepo 1996), yet 29% of the bluefin tagged in this study were located in the eastern management division. These results suggest that mixing across the 45°W management line is significantly higher than the 2–7% range estimated by ICCAT.

The presence of spawning size class fish in the mid-Atlantic, coincident with the presence of other spawning size class fish in the Gulf of Mexico and on the continental shelf off the U.S. and Canada, suggests that the distribution of adult bluefin tuna may be broader than that acknowledged by currently accepted assessments. This study is the first to validate the successful long-term (9.5 month) external attachment of self-releasing tags on giant bluefin tuna. Pop-up archival satellite tags, data-logging tags that record light levels for daily geolocation estimation and can intensively sample temperature and depth, have been succesfully tested and deployed on giant bluefin tuna in the Gulf of Maine in 1998 (M. Lutcavage, unpublished data). These new tags will allow complete reconstruction of migration routes and have the potential to make significant and rapid improvements in our understanding of migration, spawning, stock structure, and population assessments of the North Atlantic bluefin tuna. Such improvements are clearly needed for effective resource conservation and equitable fisheries allocations.

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Protective role of ascorbic acid against damage to male germ cells in rainbow trout (*Oncorhynchus mykiss*)

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Abstract: Low antioxidant levels have been implicated in damage to sperm DNA. We used a teleost fish to test if low paternal vitamin C status may cause mutations to the sperm and birth defects. During spermatogenesis, rainbow trout (*Oncorhynchus mykiss*) males were fed diets with graded levels of ascorbic acid derivative that were below and severalfold higher than those required for maximum growth. This treatment resulted in corresponding changes in levels of vitamin C in seminal plasma. We found that low levels of vitamin C in seminal plasma of rainbow trout were associated with a high percentage of abnormal embryos in the offspring. Among abnormal embryos, 34.8% were haploids or aneuploids, as revealed by flow cytometric measurement of DNA content. We found that UV-irradiated sperm gave rise to progeny with abnormalities similar to those resulting from sperm with low levels of antioxidants in seminal plasma. Ascorbic acid applied directly to semen did not prevent damage due to UV irradiation. These results provide the first experimental evidence that the lack or low levels of ascorbic acid in semen correlate with damage to male germ cells.

Résumé : De faibles concentrations d'antioxydants seraient responsables des dommages causés à l'ADN du sperme. Nous avons utilisé un téléostéen pour vérifier si l'insuffisance de la quantité de vitamine C chez le père peut causer des mutations au niveau du sperme et des malformations congénitales. Au cours de la spermatogenèse, des truites arc en-ciel (*Oncorhynchus mykiss*) mâles ont reçu des rations comportant des quantités calibrées de dérivés de l'acide ascorbique qui étaient inférieures et plusieurs fois supérieures à celles requises pour obtenir une croissance maximale. Ce traitement s'est traduit par des modifications correspondantes de la concentration de vitamine C dans le plasma séminal. Nous avons constaté qu'un niveau trop faible de vitamine C dans le plasma séminal de truite arc-en-ciel étaient associées à un pourcentage élevé d'embryons anormaux. Parmi les embryons anormaux, 34,8% étaient haploïdes ou aneuploïdes, comme l'a montré la mesure par cytométrie de flux du contenu en ADN. Nous avons observé que les descendants issus de sperme traité aux UV étaient porteurs d'anomalies similaires à celles de descendants issus de sperme à faible teneur en antioxydants dans le plasma séminal. L'addition directe d'acide ascorbique au sperme n'a pas empêché la présence de dommages dus à l'exposition aux rayons UV. Il s'agit des premières indications expérimentales montrant que l'absence ou l'insuffisance d'acide ascorbique dans le sperme est corrélée à des dommages aux cellules reproductrices mâles.

[Traduit par la Rédaction]

Introduction

Teleost fishes are the largest group of vertebrates that cannot synthesize ascorbic acid (AA), similar to some mammals, including humans, nonhuman primates, bats, and guinea pigs (Chatterjee 1973). Similar to these mammals, teleost fish do not have an active gulonolactone oxidase, the terminal enzyme in the AA synthesis pathway (Dabrowski 1990). The presence of this enzyme and vitamin C synthesis in the Actinopterygian fishes (Dabrowski 1994; Moreau et al. 1996) and Australian lungfish (Dykhuizen et al. 1980) suggests that ancestor(s) of teleost fishes lost the ability to synthesize AA. Thus, teleosts must rely on an exogenous source of vitamin C. When vitamin C is deficient, teleosts suffer classical symptoms such as scurvy (Dabrowski et al. 1990; Sandnes 1991).

During recent years, the rainbow trout (*Oncorhynchus mykiss*) has been used for studying the role of AA in male reproduction. Our previous work (Ciereszko and Dabrowski 1994) indicated that there are much higher concentrations of vitamin C in fish seminal plasma (30–60 ppm) than in blood plasma (1–10 ppm). This suggests that AA may be even more important as an antioxidant in the male reproductive tract than in blood plasma. AA in fish seminal

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plasma appeared to be well protected against oxidation by seminal plasma proteins (Liu et al. 1995). We have also established that AA concentrations in seminal plasma are regulated by dietary levels of vitamin C. AA appeared to be important for the reproduction of male fish, since its deficiency reduced sperm concentration, motility, fertility, and protein concentration and antiproteinase activity of seminal plasma of rainbow trout (Ciereszko and Dabrowski 1995; Ciereszko et al. 1996; Dabrowski and Ciereszko 1996).

There is growing evidence that AA may have a role in cell proliferation (Denis et al. 1994) and gene expression (Chojkier et al. 1989). The male germ line is much more vulnerable to mutations than the female germ line because (*i*) there are a much larger number of cell divisions in males than in females (Shimmin et al. 1993) and (*ii*) spermatozoa of rainbow trout are stored in testicular lobules or sperm ducts throughout the reproductive season, which can last up to 6 months (spermatogenesis in rainbow trout is completed about 2 months before spawning starts) (Billard 1986, 1992). Thus, in fish, the period for potential sperm DNA damage is long, as compared with animals with continuous spermatogenesis. No DNA repair capacity has been found during meiotic and postmeiotic stages of spermatogenesis or in maturing spermatozoa. As such, damage to the sperm DNA is cumulative (Inoue et al. 1993). For these reasons, rainbow trout spermatogenesis and sperm storage offers a useful model for studying the relationship between a nutrient (AA) and damage to male germ cells.

This study was undertaken to determine if dietary levels of vitamin C can influence the quality of genomic material carried by fish spermatozoa. We found that dietary treatments, resulting in low levels of vitamin C in seminal plasma, were associated with a high percentage of lethal mutations of embryos. Similar phenotypic changes in progeny were obtained by UV treatment. AA applied directly to semen did not prevent damage due to UV irradiation. These results provide the first experimental evidence that low levels of AA in semen are associated with damage to male germ cells.

Materials and methods

Effect of feeding of male rainbow trout diets supplemented with different levels of AA on quality of offspring

Three-year-old rainbow trout were maintained in six outdoor fiberglass tanks (11 fish per tank). Each tank was supplied with a constant flow of well water. Water temperature followed the natural course, decreasing from 12°C in November to about 8°C by the end of January and then increasing again to 12°C in April. Fish in groups 1–6 were fed twice daily (1% of body weight per day) starting in July 1993 with fish meal based diets supplemented with ascorbyl monophosphate magnesium salt (Showa Denko America Co., New York) at 0, 30, 110, 220, 440, and 870 ppm, respectively. These levels correspond to AA levels of 0, 10.4, 17.5, 76.8, 129.3, and 270.9 ppm, respectively (Ciereszko and Dabrowski 1995). Monophosphate ascorbyl ester was used because of its resistance to heat and diet processing and its excellent bioavailability to fish (Blom and Dabrowski 1995). Milt was collected three times during the reproductive season: December, February, and March. We sampled all available semen each time, and semen from the last sampling was used in this experiment. Four to eight males per dietary treatment were used as sperm donors in this experiment.

Milt was centrifuged at 7000 × *g* after 2–4 h of storage on ice. Trichloroacetic acid extracts for AA measurement were prepared from fresh seminal plasma. Extracts were frozen at −20°C for further analysis. Total AA concentrations in seminal plasma were determined according to Dabrowski and Hinterleitner (1989). Sperm concentration was measured according Ciereszko and Dabrowski (1993). Each semen sample was evaluated in duplicate by fertilization of 200 eggs from the same lot (pooled eggs from two females that were fed diets supplemented with vitamin C according to the recommendation of the National Research Council (1993)). A constant number of spermatozoa per egg (300 000) was employed to exclude the effect of sperm concentration on fertilization success. The quality of eggs was tested by fertilization with an excess of spermatozoa (> 3×10^6 sperm/egg) pooled from males originating from the same batch of fish as used in the experiment and raised under commercial farm conditions (London Fish Hatchery, Division of Wildlife, London, Ohio). Eggs were incubated at 12°C and survival was recorded at the eyed stage and hatching. The frequency of abnormal embryos at the eyed stage was a main endpoint in our experiment. Flow cytometry analysis of DNA content in the somatic cells of eyed embryos (10 normal and 25 abnormal) was performed as described previously (Lin and Dabrowski 1996).

Effect of UV irradiation in the presence or absence of AA on the percentage of abnormal embryos

Semen from albino rainbow trout (the only available strain during the experimental period) was shipped overnight from Egan Fish Hatchery, Bicknell, Utah. We did not observe reduced AA levels in seminal plasma of this strain. We formed three pooled semen samples, each consisting of semen from four different males. Experimental semen was supplemented with AA at 200 ppm in 40 mM KCl (to prevent sperm activation); the same amount of KCl (5% of semen volume) was added to control semen. After 4 h of preincubation on ice (to secure AA contact with sperm), sperm suspensions were exposed to UV irradiation (dose 250 mW/cm^2) for 0–6 min. Semen was stirred and kept on ice during irradiation. We then used irradiated semen for fertilization at a ratio of 500 000 spermatozoa/egg; 200 eggs were used for each fertilization. The presence of abnormal embryos was checked at the eyed stage. Flow cytometry analysis of DNA of eight abnormal embryos (timepoint 6 min, control) was performed as above.

Statistical analysis

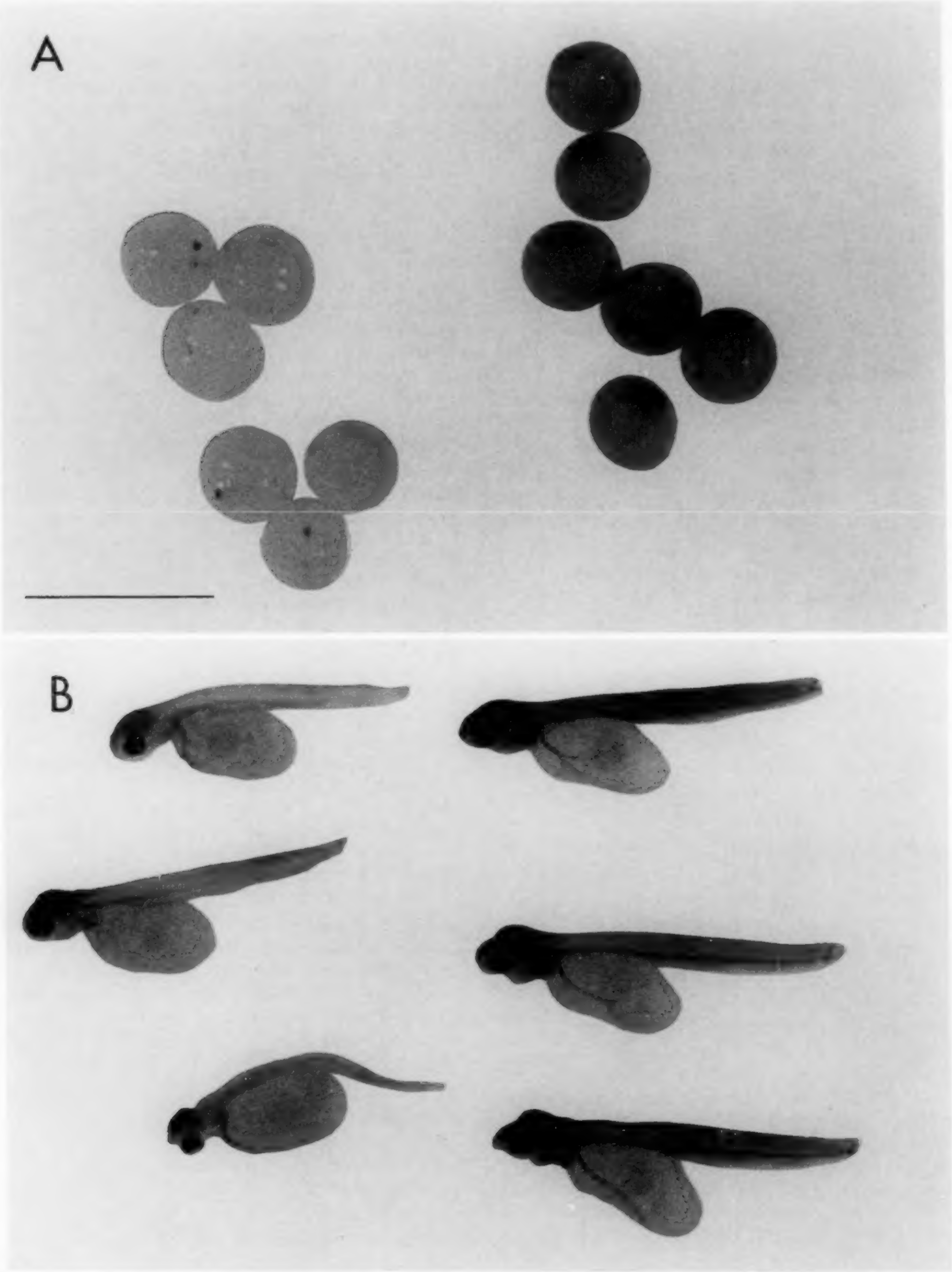
A one-way ANOVA, followed by a post hoc Fisher protected least square differences test, was used for group comparisons (StatView™ 512+, Brain Power, Inc., Calabasas, Calif.). Although dietary treatment was applied per tank, fish did eat different amounts of food with the AA and therefore were treated as separate degrees of freedom. Percentage data were arcsin transformed.

Results

Effect of feeding of male rainbow trout diets supplemented with different levels of AA on quality of offspring

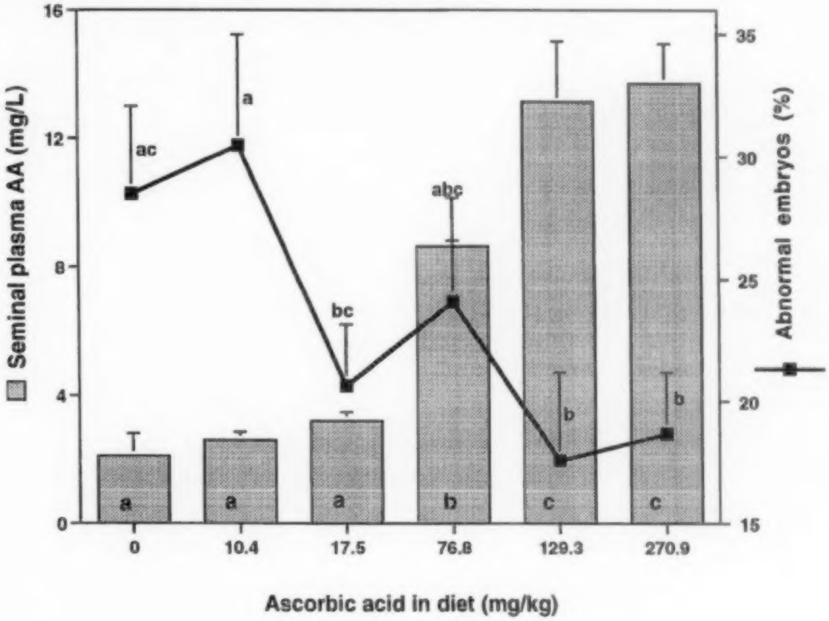
Differences in dietary intake of AA did not lead to significant differences in survival of embryos to the eyed stage. Survival rates of embryos obtained from males in six dietary treatments varied between 49.2 ± 4.33 (group 1) and 55.4 ± 4.1% (group 4). At the eyed stage, we found two groups of embryos (*i*) normally pigmented with developed eyes and (*ii*) abnormally pigmented with smaller body size and eye diameter (Fig. 1A). A significantly higher percentage of

Fig. 1. Abnormalities in rainbow trout embryos and larvae. (A) At the eyed stage, abnormal embryos (left) can be distinguished by a smaller eye diameter and a poorly developed cardiovascular system as compared with normal embryos (right). (B) Only a few larvae hatched from abnormal embryos (left); their development was impaired as compared with normal larvae (right). Scale bar = 1 mm.



abnormal embryos at the eyed stage was found in groups fed diets without AA or supplemented with a marginal dose of AA (active AA 10.4 ppm; see Ciereszko and Dabrowski 1995) than in those supplemented with higher doses of AA (Fig. 2). These embryos were still alive at this stage; however, the latter embryonic stages were impaired, as indicated by a significant negative regression between the percentages of abnormal and hatched enbryos ($y = -1.087x + 96.731$, $r^2 = 0.865$, $p < 0.001$; y = hatched embryo, %; x = abnormal embryo, %). Most of the abnormal embryos (>99%) did not

Fig. 2. Fertilization of the same pool of eggs by spermatozoa from male rainbow trout fed different levels of AA. AA concentrations in seminal plasma (bars) increased in a dose-dependent way in relation to vitamin C concentrations in the diet. The percentage of abnormal embryos (line) was higher in those from fish fed a low dose of vitamin C (10.4 mg/kg) or no vitamin C than in those from fish fed a high dose of vitamin C (129.3–270.9 mg/kg). Values are averages (mean ± SEM) of four to seven observations per dietary group (total $n = 36$ males). Means with the same letter do not differ ($P < 0.05$).



hatch. A few hatched larvae from these embryos were smaller than those hatched from normal embryos (Fig. 1B). Flow cytometry analysis of DNA content in the somatic cells of eyed embryos revealed that all normal fish were diploids whereas 34.8% of the abnormal embryos were haploids or aneuploids. It was noticeable that a relatively high percentage of abnormal embryos (21%) was recorded for controls (fertilized with an excess of semen obtained from males fed commercial diets).

Effect of UV irradiation in the presence or absence of AA on the percentage of abnormal embryos

We found that phenotypic abnormalities similar to those described above could be induced by UV irradiation. The percentage of these abnormalities correlated with the time of exposure of sperm suspensions to UV light ($y = 3.717x + 8.335$, $r^2 = 0.956$, $p < 0.01$; y = abnormal embryos, %; x = time of UV exposure, min). Analysis of cellular DNA content by flow cytometry of abnormal embryos obtained from UV irradiation revealed that 50% of them were haploids. We could not demonstrate in this experiment a protective role of AA added to seminal plasma at 200 ppm prior to UV irradiation against UV-induced sperm damage. The percentage of egg abnormalities also correlated with time of exposure of sperm suspensions, supplemented with AA, to UV light ($y = 3.799x + 9.151$, $r^2 = 0.990$, $p < 0.01$; y = abnormal embryos, %; x = time of UV exposure, min).

Discussion

This study demonstrated that supplementation of the diet of 3-year-old male rainbow trout with a high dose of vitamin C increased its concentrations within the reproductive tract and decreased the percentage of abnormal embryos in the progeny. This suggests a protective role of AA against damage to male germ cells during male gametogenesis and offers an explanation for our previous observation concerning low fertilization success of spermatozoa from fish with low vitamin C levels in seminal plasma (Dabrowski and Ciereszko 1996). Similar phenotypic abnormalities were also induced by UV irradiation. To the best of our knowledge, this is the first report providing evidence regarding the relationship between AA status in semen and embryo abnormality, caused most likely by mutations to the sperm genome. Our results extend the findings of Fraga et al. (1991) who indicated the protective effect of AA toward oxidative damage to human sperm DNA. However, these spermatozoa were not used for fertilization, so further embryo development was not tested by these authors.

A relatively high percentage of abnormal embryos (21%) was recorded for the controls. This may indicate that the eggs used were of low quality. It may be speculated that DNA repairing mechanisms within the fertilized egg could be impaired in this case and this could aggravate the manifestation of mutations in sperm cells. It has been documented that mammalian oocytes have a repair capacity for the DNA lesions present or induced in spermatozoa (Genesca et al. 1992). It would be of interest to reexamine the phenomena described in this current work of a high percentage of embryo abnormality when sperm with low AA levels in seminal plasma were used for fertilization with the eggs of females where abnormality is negligible. At present, the extent of the ability of such oocytes to neutralize sperm mutations is not known.

UV light is a known mutagenic factor. Its application leads to DNA lesions and gene mutations (Applegate and Ley 1988) or changes in the disulfide bonds of protamines and DNA–protein crosslinks (Don and Avtalion 1993). The latter changes may lead to disturbances in chromatin decondensation. In the second experiment, we investigated if UV irradiation of sperm can induce similar phenotypic changes in progeny and if exogenous AA can have any protective effect against UV irradiation. The similarity of embryo abnormalities after fertilization with the use of UV-irradiated sperm to those observed in the first experiment suggests that low AA levels in the diets are related to damage to sperm DNA. It has been shown that UV-induced DNA damage can be prevented in many cases by AA (Darr et al. 1992). We did not observe any protective effect of AA against damage to the sperm caused by UV irradiation. This suggests that the protective effect of AA observed in the earlier experiment may be related to a long-term AA effect, possibly during multiple mitotic divisions during spermatogenesis.

The role of AA in reproduction has been derived from its reducing properties and defined in terms of multiple biological actions such as cofactor required for the biosynthesis of collagen, biosynthesis of steroid and peptide hormones, and prevention or reduction of the oxidation of biomolecules (Luck et al. 1995). Embryo abnormalities due to a sperm factor recorded in our experiment may indirectly be related to the first two functions in terms of creating optimal conditions for spermatogenesis and further storage of spermatozoa. However, recent data of Fraga et al. (Fraga et al. 1991, 1996) strongly suggest that the antioxidant function may have an important role in the protection of the human sperm genome. These authors found that oxidative damage to sperm DNA (estimated from the level of 8-hydroxy-2′-deoxyguanosine, one of 20 major oxidative DNA damage products) was inversely correlated with seminal plasma AA levels. Moreover, the experimental evidence has demonstrated that the extent of sperm DNA damage may be mitigated by increased level of dietary AA. Our results suggest that the extent of damage to spermatozoa is related to the amount of vitamin C in the diet and damage to DNA of fish embryos may be related to sperm accumulated mutations.

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Increase of heart rate without elevation of cardiac output in adult Atlantic salmon (*Salmo salar*) exposed to acidic water and aluminium

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Abstract: Adult Atlantic salmon (*Salmo salar*) were exposed for 48 h to water from acidified (pH 5.2) Fossbekk River (Norway), with and without 94 µg aluminium (Al)/L added as $AlCl_3$, and to water from circumneutral (pH 6.7) Ims River (Norway) (controls). Cardiac output, heart rate, and stroke volume were monitored throughout the exposure period with Doppler flow probes placed around the ventral aorta of the fish. Fish exposed to Fossbekk River water without added Al showed few physiological disturbances. When 94 µg Al/L was added to Fossbekk River water, most of the fish died before the end of the 48-h exposure period, and a large elevation in heart rate was observed together with a decrease in plasma chloride concentrations and an increase in haematocrit, plasma glucose and plasma cortisol levels. Cardiac output was maintained at basal levels during the first 24 h of exposure because the tachycardia was accompanied by a concomitant reduction of stroke volume. Signs of arrhythmia appeared after 32 h of exposure and were associated with a further decrease in stroke volume that caused cardiac output to decrease below basal levels. The incapacity of the tachycardia to elevate cardiac output and the subsequent death of the fish suggest that this response to low pH and Al is more of a maladaptation reaction than a compensatory or adaptative reaction.

Résumé : Des saumons atlantique (*Salmo salar*) adultes ont été exposés durant 48 h à l'eau acide (pH 5,2) de la rivière Fossbekk (Norvège) avec ou sans 94 µg d'aluminium (Al)/L ajouté sous forme de $AlCl_3$ ou à l'eau neutre (pH 6,7) de la rivière Ims (Norvège) (groupe contrôle). Le débit cardiaque de même que le volume d'éjection et le rythme cardiaque des poissons ont été enregistrés tout au long de l'exposition grâce à une sonde Doppler placée autour de l'aorte ventrale. Les poissons exposés à l'eau de la rivière Fossbekk seule n'ont démontré que de légères altérations physiologiques, mais l'addition de 94 µg Al/L à l'eau de la rivière Fossbekk a entraîné la mort des poissons avant la fin des 48 h d'exposition. La mort était précédée d'une large élévation du rythme cardiaque, de même que d'une diminution de la concentration plasmatique des ions chlore et d'une augmentation de l'hématocrite et des concentrations de glucose et de cortisol plasmatique. Le débit cardiaque s'est maintenu aux niveaux de base durant les 24 premières heures d'exposition car la tachycardie était accompagnée d'une réduction du volume d'éjection. Des signes d'arrhythmie associés à une réduction plus importante du volume d'éjection sont apparus après 32 h d'exposition et ont entraîné une diminution du débit cardiaque sous son niveau de base. L'incapacité de la tachycardie à augmenter le volume d'éjection et la mort subséquente des poissons suggère que cette réponse au pH acide et à l'Al constitue une mésadaptation plutôt qu'un mode de compensation.

Introduction

Gradual losses or reductions of fish populations have been correlated with water acidification in a number of studies from Scandinavia and eastern North America (Beamish and Harvey 1972; Hesthagen and Hansen 1991). Acidification of freshwater causes a leaching of aluminum (Al) from soil and sediments (Cronan and Schofield 1979), and the mortality of fish appears to be determined primarily by Al rather than acidity (Baker and Schofield 1982).

Inorganic monomeric Al precipitates on fish gills and causes ionoregulatory and respiratory disturbances (Exley et al. 1991). Fish exposed to Al in acid water exhibit a loss of plasma ions (sodium, chloride), a decrease in blood O_2 tension and pH, and an increase in blood CO_2 tension and lactate concentration (Neville 1985; Witters 1986; Malte and Weber 1988; Sayer et al. 1991; Rosseland and Staurnes 1994). Both the ionic loss and the hypoxia associated with an exposure to Al and low pH are likely to induce compensatory responses by the cardiovascular system. Hypoxia is indeed known to modify fish heart rate and stroke volume (Randall 1982), and the ionic loss causes an increase in arterial pressure and cardiac workload that may result in a circulatory collapse (Wood 1989). Despite its major adaptive and compensatory functions, only a few studies have been concerned with the effects of Al and low pH on the fish cardiovascular system. The few studies conducted (Milligan and Wood 1982; Giles et al. 1984; Laitinen and Valtonen 1995) have also only examined heart rate, although it is known that many fish species increase their cardiac output mainly through an increase in stroke volume rather than heart rate (Farrell and Jones 1992).

The objective of the present study was to monitor cardiac output, heart rate, and stroke volume as well as the changes

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in blood chemistry of adult Atlantic salmon (*Salmo salar*) acutely exposed to Al and low pH. Rapid reductions of water pH executed in the present study can be experienced by fish during heavy rain and snowmelt (Reader and Dempsey 1989). The Atlantic salmon may be particularly affected by such acidic episodes, since it is one of the most sensitive salmonid species to Al and low pH (Rosseland and Skogheim 1984).

Methods

Experimental animals

Adult Atlantic salmon reared at the research station of the Norwegian Institute for Nature Research (NINA) in Ims, southern Norway, were used for this study. Average body weight and total length of the fish (mean ± SD) were 1518 ± 381 g and 51.5 ± 3.9 cm, respectively. Fish were starved for at least 72 h before use to avoid influence of food on blood parameters and cardiac output.

Surgical procedures

Fish were anaesthetized by immersion in an aqueous solution of clove oil (50 mg/L) as described by Anderson et al. (1997). Anaesthetized fish were placed supine in a wetted sponge operating sling and quickly fitted with a dorsal aortic cannula (PE 50, Intramedic, Clay-Adams, Sparks, Md.) as described by Smith and Bell (1964). The cannula was filled with 0.9% NaCl saline containing 25 IU heparin/mL (Apothekernes Laboratorium A.S., Oslo, Norway). After being cannulated, the fish were turned on their side and their gills were irrigated with cold water while a Doppler flow probe was installed around their ventral aorta.

To install the Doppler flow probe, the operculum and gills were gently lifted and held in an "open" position with a specially designed plastic pad. Connective tissue covering the ventral aorta was carefully teased away on a section about 4 mm long, and a cuff-type Doppler flow probe (20 MHz, Iowa Doppler Products, Iowa City, Iowa) was placed around the blood vessel. The cuffs were selected to match the diameter of the vessel. Cuffs with an internal diameter of 1.5–2.0 mm were used. The lead wire from the probe was sutured to the fish's skin at one location on the edge of the opercular cavity and at three locations on the body wall.

Experimental protocol

Immediately after surgery, fish were placed in a submerged black Plexiglas box (115 cm long × 20 cm wide × 25 cm high; 1 fish/box). The box had a black Plexiglas lid to minimize light entry and plastic grids at both ends to facilitate water circulation. Fish were allowed to recover from surgery overnight and the exposure began the morning of the following day. The physical and chemical properties of the water used in the three experimental treatments are described in Table 1. The control group was maintained in water from Ims River, a Norwegian river with circumneutral pH. A second group of fish was exposed to water from Fossbekk River, an acidified river of southern Norway, while a third group of salmon was exposed to Fossbekk River water with 94 µg Al/L added as $AlCl_3$. One flow-through tank of 1 m^3 (1000 L) containing four Plexiglas boxes was assigned to each group, so 12 fish (four per group) were exposed simultaneously during each experiment. Exposures were replicated six times between 11 October and 4 November 1997.

The fish were maintained in Ims River water for at least 72 h before the beginning of the experiment, and they were all placed back in Ims River water after the surgery and during the overnight recovery. At the beginning of the exposure, the inflow of Ims River water was turned off and replaced by an inflow of Fossbekk River water in both acid water treatment tanks (Fossbekk River water and Fossbekk River water + Al). The flow of Ims River water was maintained in the control tank. It took about 30 min for the water in the acid water treatment tanks to reach the pH of the incoming Fossbekk River water. It was only at this moment that the Fossbekk River water inflow was turned off in the high-Al concentration treatment tank, and Fossbekk River water with added Al was pumped in a closed system from a 3000-L reservoir tank. The flow of pure Fossbekk River water was maintained in the second acid water treatment tank.

Table 1. Chemistry and Al concentrations (mean ± SE) of the water used in the three treatments ($n = 8$).

Treatment	Ims River	Fossbekk River	Fossbekk + Al
pH	6.71±0.10	5.15±0.05	5.15±0.04
Temperature (°C)	8.8±0.2	7.1±0.2	10.5±0.2
Turbidity (FTU)	0.69±0.39	1.21±0.13	1.51±0.10
Color (mg Pt/L)	13.5±2.27	32.75±2.90	30.8±2.3
Conductivity (µS/cm)	72.5±0.44	48.1±1.17	49.7±0.64
Alkalinity (µequiv./L)	143±6	3.32±1.26	4.99±1.39
Ca (mg/L)	3.66±0.08	1.07±0.04	1.08±0.02
Nitrate (µg/L)	658±46	47.0±9.1	45.3±7.4
T-Al (µg/L)	50±35	237±20	330±12
Tm-Al (µg/L)	13.4±1.9	112±7	153±4
Om-Al (µg/L)	13±2	78.6±5.9	94.6±4.2
Im-Al (µg/L)	0.38±1.06	33.1±3.0	58.8±5.3
Pc-Al (µg/L)	36.6±34.1	125±18	177±13

Note: T-Al, total Al; Tm-Al, total monomeric Al; Om-Al, organic monomeric Al; Im-Al, inorganic monomeric Al; Pc-Al, polymeric colloidal Al.

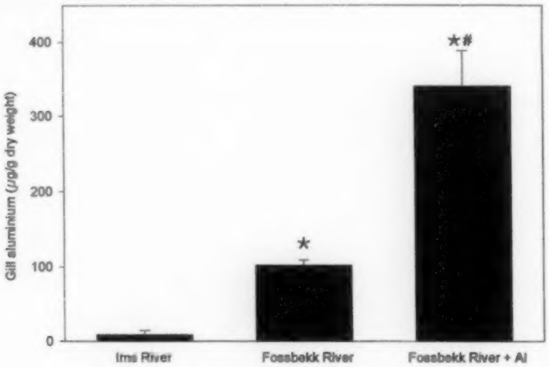
The pH was first decreased before adding Al to avoid the formation of highly toxic Al polymers that occurs when neutral water and Al-rich acid water are mixed (Verbost et al. 1995; Witters et al. 1996). To ensure that the water quality of the closed system was not degrading during the exposure, the water coming out of the holding tank was oxygenated before going back to the reservoir tank, and water from a new reservoir tank was used after 24 h of exposure. Dissolved O_2 concentrations were also monitored, and no decrease was observed during the exposure period. The water in both reservoir tanks was prepared at least 18 h before being used to give time for the different forms of bound and ionic Al to stabilize. The tanks receiving Ims River water and pure Fossbekk River water were both in a flow-through open system.

Basal levels of cardiac output and blood chemistry were measured by recording the Doppler signal for 10 min and taking a blood sample (0.3 mL) through the cannula with a 1-mL heparinized syringe immediately before the beginning of the treatment. The exposure lasted 48 h. Blood samples (0.3 mL) were taken through the cannula each 12 h, and blood flow in the ventral aorta was measured by recording the Doppler signal for 10 min each 4 h during the first 12 h of exposure, each 4 h from 24 to 36 h of exposure, and 48 h after the beginning of the exposure. Doppler signals were recorded with a pulsed Doppler flowmeter (545C-4, Department of Bioenginering, University of Iowa, Iowa City, Iowa) connected to a PCMCIA data acquisition card (DAQ-Card-AI-16E-4, National Instruments, Austin, Tex.). Cardiac output, heart rate, and stroke volume were automatically calculated from the Doppler signal for each 5 s of recording with a program written with LabView (National Instruments), and the average value for the 10-min recording period was calculated for each parameter. At the end of the exposure, the first gill arch was collected and frozen at –20°C until gill Al content was measured.

Analytical techniques

Total water Al was fractionated into organic monomeric, inor-

Fig. 1. Gill Al concentrations in Atlantic salmon exposed for 48 h to water from Ims River (n = 15), Fossbekk River (n = 19), or Fossbekk River + 94 µg Al/L (n = 15). *Significantly different from control ($p < 0.05$); #significantly different from Fossbekk River ($p < 0.05$).



ganic monomeric, and polymeric colloidal Al using a modified version of Driscoll's (1984) method. Gills were freeze-dried, weighed, and digested in a solution of HNO_3 and H_2O_2 before total Al content was measured by inductive coupled plasma emission spectroscopy. Plasma glucose was measured on whole blood immediately after sampling using a Medisense Precision QID sensor, and haematocrit was read after centrifugation of blood in capillary tubes (5 min, 3500 rpm). The plasma of the remaining blood was separated by centrifugation (5 min, 5000 rpm) and frozen. Plasma chloride concentrations were measured by coulometric titration using a Radiometer CMT 10 chloride titrator. Total plasma cortisol concentrations were estimated by radioimmunoassay as described by Simensen et al. (1978) and modified for fish by Olsen et al. (1992). The detection limit of the assay was 1.33 ± 0.73 nmol/L. Intraassay variation was less than 4.2%, interassay variation was less than 8.3%, and nonspecific binding varied from 0.6 to 2.0% of the total activity. Recovery was 93, 96, 100, and 97% at 4, 17, 34, and 69 nmol cortisol/L, respectively.

Statistical analysis

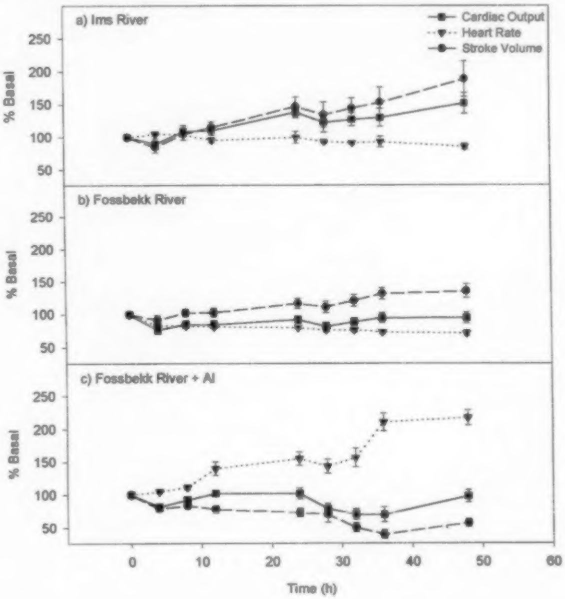
For each parameter measured, the statistical significance of the variation in time observed within a group was tested using the univariate test for repeated measurements in the PROC GLM function of the SAS statistical package. The significance levels were determined using F values adjusted with the Huynh and Felt epsilon to protect against a violation of the assumption of compound symmetry. Gill Al contents were compared using the Kruskall–Wallis test and Dunn's test for multiple comparisons.

Results

Gill Al content

The amount of Al measured in the gills of the fish after 48 h of exposure (Fig. 1) was related to the concentration of Al present in the water (Table 1). Fish exposed to Fossbekk River water + 94 µg Al/L had significantly more Al in their gills than fish exposed to both Ims River water and Fossbekk River water only, and the gill Al content of fish exposed to Fossbekk River water was significantly higher than the gill Al content of fish exposed to Ims River water.

Fig. 2. Cardiac output, heart rate, and stroke volume in Atlantic salmon exposed for 48 h to (a) Ims River water (n = 13), (b) Fossbekk River water (n = 13), or (c) Fossbekk River water + 94 µg Al/L (n = 17).



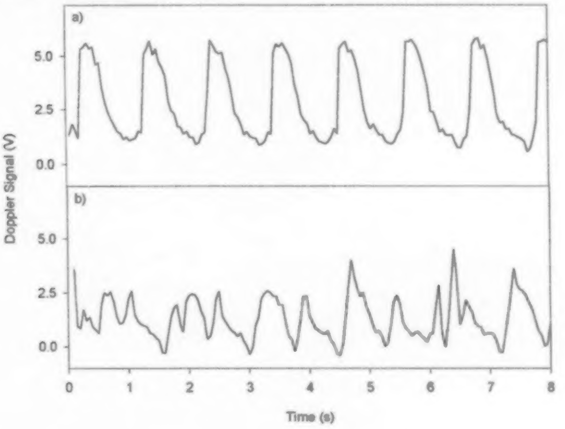
Heart function

Basal values of cardiac output, heart rate, and stroke volume (mean ± SE) were 10.6 ± 1.8 $mL{\cdot}min^{-1}{\cdot}kg^{-1}$, 46.6 ± 2.7 beats/min, and 0.24 ± 0.02 mL/kg, respectively. Cardiac output of the fish from the control group (Ims River water) increased gradually (p = 0.0001) during the exposure, reaching a maximum of 152% of basal level after 48 h of exposure (Fig. 2a). This increase in cardiac output resulted entirely from an increase in stroke volume (189% of basal level after 48 h, p = 0.0001), since heart rate showed a slight but significant (p = 0.0008) decrease throughout the exposure period (85% of basal after 48 h).

Fish exposed to Fossbekk River water exhibited a slight decrease in cardiac output to about 80% of basal level 4 h after the beginning of the exposure (Fig. 2b). This decrease in cardiac output was mainly the result of a reduction in heart rate. Stroke volume increased gradually afterward (p = 0.0001), reaching 125% of basal level after 48 h of exposure. The increase in stroke volume did not, however, significantly increase cardiac output (p of time effect = 0.1449), since heart rate continued to decrease during the rest of the exposure period (p = 0.0001), reaching 66% of basal level after 48 h of exposure.

Only three of the 17 fish exposed to Fossbekk River water + Al survived the total 48-h exposure period. The majority of the fish died between 36 and 48 h of exposure. An increase in heart rate (140–150% of basal level) was apparent 12 h after the beginning of the exposure (p of time effect = 0.0001) (Fig. 2c), but cardiac output remained at basal values because stroke volume was reduced to 75% of basal level (p of time effect = 0.0001). After 32 h of exposure

Fig. 3. Doppler signal of an individual Atlantic salmon (*a*) before the beginning of the exposure (heart rate = 53.3 beats/min) and (*b*) after 36 h of exposure to Fossbekk River water + 94 µg Al/L (heart rate = 102.9 beats/min). Signs of arrhythmia are clearly visible toward the end of the exposure.



(i.e., about 4 h before fish typically started to die), signs of arrhythmia appeared (Fig. 3) and resulted in a further increase in heart rate to 200% of basal level after 36 h of exposure. Cardiac output, however, decreased to 70% of basal level (*p* of time effect = 0.0191) because of a concomitant reduction of stroke volume to 40% of basal level (Fig. 2*c*).

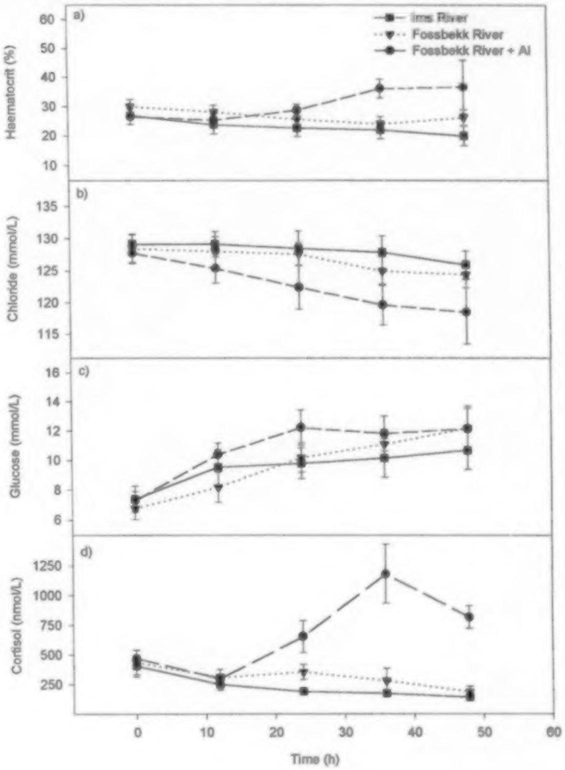
Blood chemistry

Haematocrit (Fig. 4*a*) of the fish exposed to Fossbekk River water + Al was greatly increased (*p* of time effect = 0.0005) after 36 and 48 h of exposure (about 37% after 36 and 48 h of exposure compared with 27% at the beginning of the exposure). In contrast, the haematocrit of the fish exposed to Ims River water (controls) and Fossbekk River water decreased slightly (*p* = 0.0138 and 0.0427, respectively) throughout the exposure period due to repetitive blood sampling (from 27 to 20% in Ims River water and from 30 to 26% in Fossbekk River water).

Plasma chloride concentrations (Fig. 4*b*) of fish exposed to Fossbekk River water and Fossbekk River water + Al showed a significant decrease throughout the exposure period (*p* = 0.0052 and 0.0003, respectively). The loss of plasma chloride was greater when Al was added: from 128 to 119 mmol/L in Fossbekk River + Al and from 128 to 124 mmol/L in Fossbekk River water alone. Plasma chloride concentrations of fish exposed to Ims River water (controls) did not vary significantly with time (*p* of time effect = 0.3410).

All treatments caused significant increases in plasma glucose concentrations (Fig. 4*c*). Plasma glucose of fish exposed to Fossbekk River water + Al reached 12 mmol/L after 24 h of exposure (*p* of time effect = 0.0001) and remained stable at this level until the end of the exposure. Plasma glucose also reached 12 mmol/L (*p* of time effect = 0.0006) in fish exposed to Fossbekk River water alone, but the increase was slower in these fish, and this concentration was only reached after 48 h of exposure. Plasma glucose concentrations of fish exposed to Ims River water (controls) remained elevated at 8–9 mmol/L throughout the exposure period (*p* of time effect = 0.0002).

Fig. 4. (*a*) Haematocrit, (*b*) plasma chloride, (*c*) plasma glucose, and (*d*) plasma cortisol in Atlantic salmon exposed for 48 h to water from Ims River (*n* = 13), Fossbekk River (*n* = 11), or Fossbekk River + 94 µg Al/L (*n* = 11).



Plasma cortisol concentrations (Fig. 4*d*) of fish exposed to Fossbekk River water + Al decreased during the first 12 h of exposure (from 476 to 312 nmol/L) but subsequently exhibited a substantial increase, reaching a maximum of 1088 nmol/L after 36 h of exposure (*p* of time effect = 0.0084). Plasma cortisol concentrations of fish exposed to Fossbekk River water decreased in a similar way during the first 12 h of exposure, remained stable at about 315 nmol/L from 12 to 24 h of exposure, and then kept on decreasing to reach 187 nmol/L at the end of the exposure (*p* of time effect = 0.0014). In fish exposed to Ims River water (controls), plasma cortisol decreased from 408 to 192 nmol/L during the first 24 h of exposure but only decreased from 192 to 141 nmol/L during the second half of the exposure (*p* of time effect = 0.0146).

Discussion

Adult Atlantic salmon were exposed to low pH and Al for 48 h to examine the compensatory adaptations exhibited by the heart when ionoregulatory and respiratory disturbances are induced. Exposure to both Fossbekk River water and Fossbekk River water + 94 µg Al/L created a dose-dependent accumulation of Al on fish gills. However, major physiological alterations were only observed in the high-Al treatment, and the exposure to Fossbekk River water alone caused only minor disturbances.

Haematocrit and plasma chloride slightly decreased with time in fish exposed solely to Fossbekk River water, but these effects are likely attributable to repetitive blood sampling, since they were also observed in the control group. Cardiac output was also decreased below basal levels during the entire exposure, but this was most likely related to the fact that Fossbekk River water temperature was always 2°C lower than Ims River water, which was present during the recording of basal levels. A reduction in water temperature is indeed known to cause a decrease in cardiac output through a lowering of heart rate (Farrell and Jones 1992), and Laitinen and Valtonen (1995) observed a similar increase in the heart rate of brown trout (*Salmo trutta*) when water temperature was increased by only 3°C.

Fish exposed to Fossbekk River water also differed from the control group (Ims River) by the absence of a large increase in cardiac output during the last 24 h of exposure. This increase in cardiac output in the control group most likely indicates that the holding system was causing discomfort to the fish and inducing a stress response. Indeed, although plasma cortisol levels decreased during the first 24 h of exposure in the control group, they afterward stabilized at about 170 nmol/L, which is much higher than the basal levels typically observed in salmonids (Wendelaar Bonga 1997). The time course of plasma cortisol probably illustrates an initial recovery from the major stress of surgery followed by the maintenance of a lower level of stress caused by the confinement of the fish in the Plexiglas boxes. The maintenance of elevated plasma glucose levels is additional evidence of a stress response in the control group.

The smaller amplitude of the increase in cardiac output and stroke volume observed in the fish exposed to Fossbekk River water only may be due to the slightly colder temperatures (about 2°C lower) of this river, since, as mentioned previously, the difference in temperature was important enough to cause a reduction in cardiac output. There is indeed no reason to believe that the fish exposed to Fossbekk River water were less stressed than the fish exposed to Ims River water (controls), since their plasma cortisol and glucose levels were slightly higher than those of the control group. The presence of higher levels of plasma cortisol and glucose in the fish exposed to Fossbekk River water indicates that the acid water created some additional stress to the fish.

Thus, Fossbekk River water alone caused only minor disturbances to the fish, although it contained 237 μg Al/L. The low toxicity of the Al is most likely related to the high dissolved organic matter content of Fossbekk River (as suggested by water color), which binds Al and reduces the proportion of toxic ionic species. However, when 94 μg Al/L was added to Fossbekk River water, the concentration of toxic inorganic monomeric Al was raised enough to greatly increase the toxicity of the acidic water and induce death within 48 h in most of the fish. Death was preceded by large increases in plasma glucose and cortisol levels (Fig. 4) typical of acute exposures to toxic chemicals (Fu et al. 1990; Hontela et al. 1996). An increase in haematocrit and a reduction in plasma chloride concentrations were also observed, indicating an alteration of ionoregulatory mechanisms. Heart rate was increased considerably, but cardiac output remained stable and even decreased toward the end of the exposure because of a reduction in stroke volume. Similar increases in heart rate have been previously observed in rainbow trout (*Oncorhynchus mykiss*, previously *Salmo gairdneri*) and brown trout exposed to acid water with or without Al (Milligan and Wood 1982; Giles et al. 1984; Laitinen and Valtonen 1995). This is, however, the first study to show that cardiac output itself does not increase during an exposure to low pH and Al, in spite of increased heart rate.

In fish, an increase in heart rate is an inefficient way to elevate cardiac output because the reduction in filling time and force of contraction involved provoke a reduction in stroke volume (Farrell and Jones 1992). The induction of tachycardia is thus a rather strange strategy for a fish to cope with the increase in metabolic rate observed by others during an exposure to acidity and Al (Neville 1985; Malte 1986; Wood and McDonald 1987), since the fish need to increase their cardiac output to maintain an elevated metabolic rate. Fish exposed to acid water and Al have also been shown to experience hypoxia (Malte 1986; Malte and Weber 1988; Playle et al. 1989), and the typical response of a fish to hypoxia is usually a bradycardia rather than a tachycardia. The bradycardia results in a longer residence time of the blood in the gill lamellae, which allows the diffusion of oxygen into the blood to proceed further towards completion (Satchell 1991).

As the heart was trying to maintain tachycardia in hypoxic conditions, blood pressure and cardiac workload must have increased because of the elevation in blood viscosity associated with the increase in haematocrit observed (Milligan and Wood 1982). The demand on the heart must then have been very important, and the appearance of arrhythmia a few hours before death likely resulted from myocardial hypoxia brought about by the maintenance of tachycardia under hypoxic and high blood pressure conditions. Associated with the appearance of arrhythmia was a further decrease in stroke volume and a reduction in cardiac output below basal levels, which eventually led to cardiac failure.

It is thus possible to question the benefits obtained by the fish from the tachycardia observed, and this phenomenon even appears to illustrate more of a maladaptation reaction than a compensatory or adaptative reaction. Milligan and Wood (1982) showed that the increase in heart rate was the result of increased adrenergic stimulation to the heart and not a withdrawal of vagal cholinergic tone in rainbow trout exposed to low pH without Al. A similar increase in heart rate was also observed in rainbow trout exposed to a lethal concentration of copper and was proposed as part of a general acute toxicity syndrome that may be common to many toxic metals (Wilson and Taylor 1993).

In conclusion, this study has shown that the exposure to water from the acidified Fossbekk River caused few physiological disturbances to adult Atlantic salmon but that the addition of 94 μg Al/L led to death within 48 h. Death was preceded by an increase in plasma glucose, cortisol, and haematocrit, together with a reduction in plasma chloride concentrations and an arrhythmia. The arrhythmia caused cardiac output to decrease below basal levels and followed a long-lasting tachycardia during which cardiac output was maintained at basal levels.

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Linear model analysis of net catch data using the negative binomial distribution

James H. Power and E. Barry Moser

Abstract: Sampling with nets or trawls remains a common technique for determining the comparative abundances of aquatic organisms, and the objective of such studies is frequently to evaluate relationships among the counts of individuals caught and exogenous variables. Analysis of such data is often done with a general linear model (e.g., ANOVA, ANCOVA, regression), assuming an underlying normal probability distribution. Such analyses are not fully satisfactory because of the symmetry and continuous nature of the assumed normal probability distribution and the high variance to low mean value relationships common to counts of biological populations. The negative binomial is a discrete probability distribution that is recognized as a suitable descriptor of organism count data. We present an approach for undertaking linear model analyses of net catch data that permits estimation of model parameters (including the negative binomial *k* parameter) and hypothesis testing of both continuous and discrete model effects and their interactions using bootstrap replication. The analysis incorporates adjustment for varying element sizes, such as differences in the amounts of water filtered during sampling.

Résumé : L'échantillonnage au filet droit ou au chalut reste une technique couramment utilisée pour mesurer et comparer l'abondance des organismes aquatiques, et l'objectif de ces études est souvent d'évaluer les relations entre les effectifs des individus capturés et certaines variables exogènes. L'analyse de ces données se fait souvent à l'aide d'un modèle linéaire général (p.ex. analyse de variance, analyse de covariance, régression) supposant à la base une distribution normale des probabilités. De telles analyses ne sont pas parfaitement satisfaisantes à cause de la symétrie et de la nature continue de la distribution normale supposée des probabilités, et des relations entre la forte variance et la faible valeur moyenne qui caractérisent les dénombrements des populations vivantes. La distribution binômiale négative est une distribution discrète qui est considérée comme un descripteur acceptable des données de dénombrement des organismes. Nous présentons un mode d'analyse par modèle linéaire des données sur les prises au filet qui permet d'estimer les paramètres du modèle (y compris le paramètre *k* de la distribution binômiale négative) et de vérifier l'hypothèse sur les effets à la fois continus et discrets du modèle et leurs interactions avec la réplication par bootstrap. L'analyse intègre un ajustement pour des tailles variables des éléments, comme des différences dans les quantités d'eau filtrées pendant l'échantillonnage.

[Traduit par la Rédaction]

Introduction

Sampling with nets or trawls remains one of the most common techniques for determining the comparative abundances of aquatic organisms. Numerous investigators have confronted the difficulties of interpreting the data produced from such sampling, and there has been extensive consideration of the problem (Smith and Richardson 1977; Fasham 1978; Pennington 1983, 1986, 1996; Jahn and Smith 1987; Smith 1990; Cyr et al. 1992; Smith 1993). Frequently the investigator wishes to use a collection of net samples to evaluate possible relationships between abundance of a particular organism and one or more exogenous variables. Examples of hypothesized explanatory variables might include depth strata, bottom type, day–night differences, abundances of other taxa, tidal currents, fronts, or the values of water quality parameters such as temperature. Often, these variables are observed in combination, resulting in the possibility of interactions amongst them.

Testing hypotheses about such relationships requires a statistical analysis of the counts of organisms caught by the gear. Unless nonparametric approaches are used, such analyses commonly suffer from lack of a logical and defensible statistical framework, especially with regard to assumptions about the statistical distribution describing the data. Often the raw, or logarithmically transformed, counts of organisms caught are analyzed using a general linear model analysis (regression, ANOVA, or ANCOVA) with the assumption that the data, or logarithmically transformed data, are normally distributed. This is due to both the widespread familiarity with such analyses and the ready availability of suitable software. The practice is commonplace, and careful consideration of the implications of using the underlying normal probability distribution is rare.

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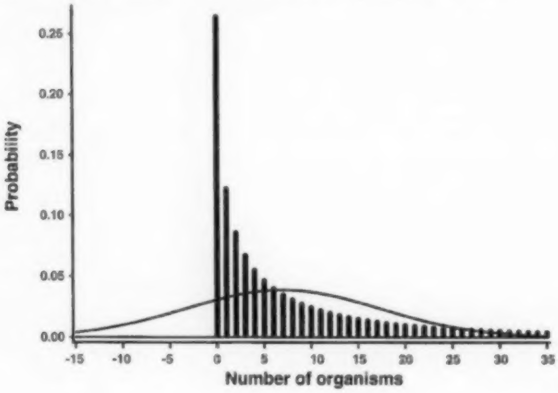
Table 1. Nonlinear least squares analysis of the ichthyoplankton data set compiled by Cyr et al. (1992) using forms of the general variance to mean relationship $s^2 = g\bar{x} + h\bar{x}^l$ as described by Perry and Woiwood (1992).

	Parameter				
Model^a	g	h	l	Split	Residual sum of squares
Nelder (3)	1.000	2.267	1.887		8.542×10^{12}
Taylor (4)	0.000	2.289	1.886	0.39	8.542×10^{12}
Iwao (5)	11.774	1.069	2.000	−10.08	8.552×10^{12}
Negative binomial	1.000	1.085	2.000		8.552×10^{12}

Note: Only mean ichthyoplankton catches of <1000 animals in the Cyr et al. (1992) data set were used because the inclusion of larger mean catches tended to dominate the parameter estimates. The Taylor and Iwao models were formulated as split domain models, and the variance was considered to be equal to the mean at mean values below the split point.

^{a}Number in parentheses after model name corresponds to model number presented by Perry and Woiwood (1992).

Fig. 1. Comparison of the normal probability distribution with the negative binomial probability distribution. Both distributions were computed using the results of Cyr et al. (1992), and both have a mean value of 7 organisms and a variance of 93.4.



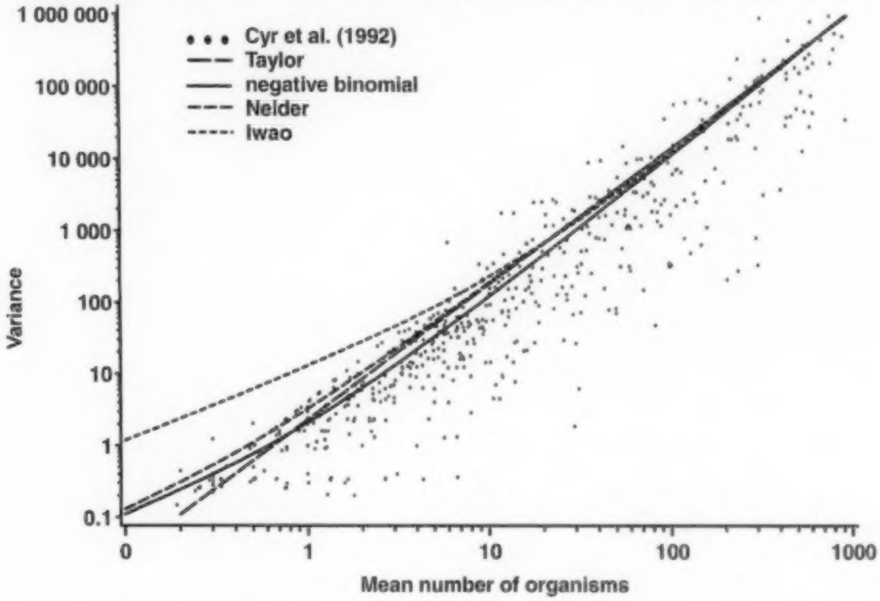
The assumption that a normal probability distribution underlies the observed data can be questioned for several reasons. First, the normal distribution represents probabilities associated with a continuous variate that can theoretically take on any possible value within a plausible range, including fractional values if the observational method has the necessary precision. Net catch data are discrete counts of the organisms that were collected in the samples and can only be represented by nonnegative integers. For example, assuming a normal distribution implicitly assigns a probability to the collection of, say, 7.1–7.9 animals in a sample. Normalizing the data to a standard sampling element, such as the volume of water filtered, may make it appear to be continuous, but in reality, only a finite subset of observed values are possible, depending on the size of the sample element. Net catch data are also characterized by low mean values and high variances. In this event, because of its symmetry, the normal probability distribution can imply a substantial probability of catching a negative number of animals (Fig. 1). Finally, equality of variances among treatments is assumed in general linear model analyses, and no relationship between mean values and the variance of the data is assumed.

The negative binomial is a discrete probability distribution that has received wide recognition as being a suitable descriptor of animal abundance data (Smith and Richardson 1977; Zweifel and Smith 1981; Jahn 1987; Jahn and Smith 1987; Welch and Ishida 1993; White and Bennetts 1996). It is general, including both the Poisson and geometric distributions as special cases, and it further reflects actual observed data in that the modal number of organisms caught may be zero, with catches of one or more organisms having monotonically decreasing probabilities (White and Bennetts 1996) (Fig. 1).

The negative binomial distribution also conforms to the accumulated empirical evidence that the variance of plankton net catch data almost invariably exceeds the mean value. The expression of sample variance as a power function of mean abundance has been termed Taylor's power law (Taylor 1961). Cyr et al. (1992) conducted an extensive literature review of ichthyoplankton sampling and summarized the data to relate the variance to mean organism abundance with the power relationship $s^2 = 3.61\bar{x}^{1.74}$. The ichthyoplankton data set of Cyr et al. (1992) (generously provided to us by those authors) was reanalyzed as in Perry and Woiwood (1992) by using variations of the general model $s^2 = g\bar{x} + h\bar{x}^l$. The negative binomial distribution relates the variance to the mean as $s^2 = \bar{x} + \bar{x}^2/k$, so that for the negative binomial, $g = 1$, $h = 1/k$, and $l = 2$. Nonlinear least squares was used to determine the values of g, h, and l that minimized the residual sum of squares. Table 1 and Fig. 2 demonstrate that all four varince–mean models, including that defined by the negative binomial, fit the observed ichthyoplankton catch data about equally well with respect to the residual sum of squares. Only the Iwao model yields a visually unsatisfactory fit to the data. Such fits of variance–mean relationships for animal abundance data can be viewed as a broad "rule of thumb," and, of course, individual circumstances can deviate greatly from the fitted curves. Nonetheless, they are useful for roughly anticipating the magnitude of the variance that might be associated with a given abundance, and indeed, Cyr et al. (1992) applied it in this sense to approximate the statistical power that might be associated with a planned ichthyoplankton survey.

It appears from the preceding that the negative binomial is a reasonable probability distribution for the overall description of net catch data. Unfortunately, the lack of a suitable analytical approach has precluded the use of the negative binomial for the next appropriate application: as the underlying distribution in analyses of net catch data relative to sets of exogenous variables. Such a methodological approach is described here, patterned after the "generalized linear model" approach that was first described by Nelder and

Fig. 2. Nonlinear regression fits of the Cyr et al. (1992) data. Fitted curves correspond to those models examined by Perry and Woiwood (1992) and were computed using the nonlinear least squares parameter estimates provided in Table 1.



Wedderburn (1972) and is further described in McCullagh and Nelder (1989) and Dobson (1990). Generalized linear models have the same basic structure as the more familiar general linear model (which assumes normality) but allow for a wider variety of underlying probability distributions (including the negative binomial if the k parameter is held constant). The negative binomial linear model will hereafter be referred to as the NBLIM approach.

Model specification

We start with the negative binomial parameterization given by McCullagh and Nelder (1989, p. 373):

$$\Pr(Y = y; \alpha, k) = \frac{(y+k-1)!}{(k-1)!y!}\,\frac{(\alpha)^y}{(1+\alpha)^{(y+k)}}$$

with mean

$$E(Y) = \mu = k\alpha$$

and variance

$$\operatorname{var}(Y) = k\alpha + k\alpha^2 = \mu + \frac{\mu^2}{k}\cdot$$

The negative binomial parameter k complicates the analysis and is discussed later.

A concern when dealing with net catch data is the variation among samples in the amount of water filtered (or the distances or durations of tows). We assume that we have collected n samples, indexed by the subscript i. We take into account the variations in sample element size by following Bissell (1972) and define w_i as the ratio of the water filtered during the ith tow to the overall mean volume of water filtered on all tows, regardless of covariates. The same approach can be used if the sample effort is described by distance or duration of tow. The probability of catching y animals in a net then becomes

$$\Pr(Y = y; \alpha, k) = \frac{(y+k-1)!}{(k-1)!y!}\,\frac{(w_i\alpha)^y}{(1+w_i\alpha)^{(y+k)}}$$

$$= \frac{\Gamma(y+k)}{\Gamma(k)\Gamma(y+1)}\,\frac{(w_i\alpha)^y}{(1+w_i\alpha)^{(y+k)}}$$

where $\Gamma(\cdot)$ is the gamma function.

We use maximum likelihood estimation to obtain a set of model parameters for hypothesis tests about the role of exogenous variables. For a collection of n net samples, we wish to find parameters that maximize the log-likelihood function

$$l(\mu; \mathbf{y}, k)$$

$$= \sum_{i=1}^{n}\left[\ln\left(\frac{\Gamma(y_i+k)}{\Gamma(k)\Gamma(y_i+1)}\right) + y_i \ln\left(\frac{w_i\alpha}{(1+w_i\alpha)}\right) - k\ln(1+w_i\alpha)\right]$$

If we let $\alpha = \mu/k$, then

$$l(\mu; \mathbf{y}, k)$$

$$= \sum_{i=1}^{n}\left[\ln\left(\frac{\Gamma(y_i+k)}{\Gamma(k)\Gamma(y_i+1)}\right) + y_i \ln\left(\frac{w_i\mu}{(1+w_i\mu)}\right) - k\ln\left(\frac{k+w_i\mu}{k}\right)\right]$$

We define a linear model with a total of p parameters β_j and covariates x_{ij}:

$$\eta_i = \sum_{j=1}^{p} x_{ij}\beta_j.$$

The covariates x_{ij} can be continuous, or they may represent nominal (classification) variables through the use of dummy, or indicator, variables. For example, if nets were deployed at three depth strata (say, surface, midwater, and bottom), then two covariates x_{i2} and x_{i3} can be defined so that $x_{i2} = x_{i3} = 0$ for surface samples, $x_{i2} = 1$ and $x_{i3} = 0$ for midwater samples, and $x_{i2} = 0$ and $x_{i3} = 1$ for bottom samples. The covariate x_{i1} and associated parameter β_1 are used for the "intercept" term in the model, and $x_{i1} = 1$ for all samples. A further set of covariates for, say, day–night differences can be added to the model using $x_{i4} = 0$ for daytime samples and $x_{i4} = 1$ for nighttime samples. Interaction between depth and light is further defined by taking the products of x_{i2} and x_{i3} with x_{i4}, e.g., $x_{i5} = x_{i2}x_{i4}$, and $x_{i6} = x_{i3}x_{i4}$. Further examples of the use of indicator variables are in many statistics texts such as Neter et al. (1990).

The value of η_i is related to the likelihood (l) using a link function, and the investigator has a choice of which link function to use. The canonical link function for the negative binomial is (McCullagh and Nelder 1989)

$$\eta_i = \sum_{j=1}^{p} x_{ij}\beta_j = \ln\left(\frac{\mu_i}{\mu_i + k}\right)$$

so that μ_i can be written

$$\mu_i = \left(\frac{k\exp\left(\sum_{j=1}^{p} x_{ij}\beta_j\right)}{1 - \exp\left(\sum_{j=1}^{p} x_{ij}\beta_j\right)}\right) = \frac{k\exp(\eta_i)}{1 - \exp(\eta_i)}$$

and the right-hand side of the above expression can be substituted for μ in the likelihood function. Another choice of link function is the logarithmic link

$$\eta_i = \sum_{j=1}^{p} x_{ij}\beta_j = \ln(\mu_i)$$

so that

$$\mu_i = \exp\left(\sum_{j=1}^{p} x_{ij}\beta_j\right) = \exp(\eta_i).$$

In some cases, it may be appropriate to consider that the k parameter also depends on the covariates x_{ij}. One way to incorporate this possibility is to express the value of the particular k_i's as another linear sum of the covariates, i.e.:

$$k_i = \sum_{j=1}^{p} x_{ij}\kappa_j.$$

In practice, this would be most useful when the x_{ij} represent indicator variables for discrete treatments. This approach is then similar to that of White and Bennetts (1996), who allowed for k and μ to vary among treatments but did not allow for varying sample element sizes.

By incorporating the above expressions for μ_i and k_i, the log-likelihood when the canonical link is used can be written

$$l = \sum_{i=1}^{n}\left[\ln\left(\frac{\Gamma(y_i + k_i)}{\Gamma(k_i)\Gamma(y_i + 1)}\right) + y_i\left(\eta_i + \ln\left(\frac{w_i}{1 - [\exp(\eta_i)(1 - w_i)]}\right)\right) + k_i \ln\left(\frac{1 - \exp(\eta_i)}{1 - [\exp(\eta_i)(1 - w_i)]}\right)\right]$$

The log-likelihood when the logarithmic link is used is

$$l = \sum_{i=1}^{n}\left[\ln\left(\frac{\Gamma(y_i + k_i)}{\Gamma(k_i)\Gamma(y_i + 1)}\right) + y_i\left(\eta_i + \ln\left(\frac{w_i}{k_i + w_i\exp(\eta_i)}\right)\right) + k_i \ln\left(\frac{k_i}{k_i + w_i\exp(\eta_i)}\right)\right]$$

Parameter estimation

The log-likelihood is maximized when the elements of the gradient vector are equal to zero, i.e., $\partial l/\partial\kappa_j = 0$ and $\partial l/\partial\beta_j = 0$, so we need to find the set of parameters κ_j and β_j that make these conditions true for the data set under consideration (full expressions for the gradient terms are provided in the Appendix). McCullagh and Nelder (1989) described an iterative, weighted linear regression algorithm (the scoring method) that can be used to find parameter estimates in many generalized linear models. However, the unknown parameters κ_j in the negative binomial distribution prevents the use of the scoring method. The scoring method is a modification of the Newton–Raphson root-finding algorithm, and a solution to the NBLIM model can be obtained using the classical Newton–Raphson algorithm (Dobson 1990).

Initial starting estimates for κ_j and the β_j are needed for the Newton–Raphson iterative algorithm. These can be obtained by assuming that all parameters with the exception of those associated with the "intercept" (κ_1 and β_1) are zero (i.e., κ_j, $\beta_j = 0$ for $j = 2,..., p$), setting $\beta_1 = \mu$, the mean of all the data, and using an initial estimate for κ_1, the approximation provided by Anscombe (1950) as $\kappa_1 = \frac{\bar{x}^2}{s^2 - \bar{x}}$.

Statistical inference

There are several approaches for testing whether there are significant effects associated with the estimated parameters. One is to use the log-likelihood ratio statistic to compare the goodness-of-fit of a model with a larger number of parameters with one with a reduced set of parameters to determine if the reduced model still adequately describes the data. A saturated model with n parameters will exactly fit a set of n observations (here, we are not including k in the count of n parameters), and the log-likelihood associated with such a model is $l(\boldsymbol{\beta}_{\max};\mathbf{y})$. The log-likelihood ratio statistic for comparing such a model to one with fewer parameters is defined as $\ln \lambda = l(\mathbf{b}_{\max}; y) - l(\mathbf{b}; y)$ and the scaled deviance (D) is twice the difference between the log-likelihood for the saturated model and one with fewer parameters, e.g., $D = 2\ln\lambda = 2[l(\mathbf{b}_{\max}; y) - l(\mathbf{b}; y)]$. If the reduced model fits the data nearly as well as the one with n parameters, then $D \sim \chi^2_{n-p}$. We conclude that the fit of the reduced model is poor if D exceeds the critical value for χ^2 with $n - p$ degrees of freedom.

Hypothesis tests concerning the effects of factors, continuous covariates, and any interactions are done similarly by comparing a model with, say, p parameters with a reduced model (with q parameters) where some of the parameters (β_j) associated with a subset of the covariates are hypothesized to be zero. For example, assume that two models are to be compared, one having the parameters associated with a model of just the main effects (having q parameters) and one associated with a model having the main effects plus their interaction terms (and having p parameters). The null hypothesis H_0 is that $\beta_j = 0$ for the interaction terms $i = q + 1$ to p. If D_0 is the scaled deviance (twice the log-likelihood ratio) for the model with q parameters and D_1 is the scaled deviance for the model with p parameters, then if both models fit the data well, $D_1 \sim \chi^2_{n-p}$ is equivalent to $D_0 \sim \chi^2_{n-q}$, and the model associated with D_0 is preferable because it has fewer parameters yet adequately describes the data. It can be shown that the difference between the two deviances is $D = D_0 - D_1 = 2[l(\boldsymbol{b}_1; \boldsymbol{y}) - l(\boldsymbol{b}_0; \boldsymbol{y})] \sim \chi^2_{p-q}$ (Dobson 1990), where $l(\boldsymbol{b}_1; \boldsymbol{y})$ and $l(\boldsymbol{b}_0; \boldsymbol{y})$ are the log-likelihoods associated with the two models and computed using the methodology of the preceding section. If D is greater than the critical value of χ^2_{p-q}, then we can reject H_0 and conclude that there is interaction between the factors described by the covariates.

White and Bennetts (1996) used the log-likelihood ratio in their discussion of negative binomial model selection. However, they additionally discussed the use of Aikaike's Information Criteria (AIC) as a supplemental tool to aid in model selection. For the two models discussed above, with p and q parameters, the AIC values are defined as $-2l(\boldsymbol{b}_1; \boldsymbol{y}) + 2p$ and $-2l(\boldsymbol{b}_0; \boldsymbol{y}) + 2q$, and one would choose the model with the lowest AIC value as being the most parsimonious model.

An alternative to the log-likelihood ratio or AIC for making inferences is to use the Wald statistic (Dobson 1990). One might hypothesize that particular values of β_j are zero and compute the Wald statistic as $(\mathbf{b} - \boldsymbol{\beta})' \mathscr{L}(\mathbf{b} - \boldsymbol{\beta})$. $\mathscr{L}$ is the information matrix, available as the negative of the second derivatives of the log-likelihood function with respect to the parameter estimates, i.e., the Hessian matrix computed as part of the Newton–Raphson algorithm. For large samples the Wald statistic is approximately distributed as χ^2_p.

A concern when using log-likelihood ratios to evaluate parameter effects is the assumption that the statistic is distributed as χ^2. This assumption can be avoided by using the bootstrap method to obtain replicate estimates of each of the model parameters (Efron and Tibshirani 1993). In this approach, one repeatedly samples, using a pseudorandom number generator, the observed data with replacement. It should be emphasized that when using the bootstrap to analyze factorial designs, having nominal covariates, subsets of replicate samples should be drawn from each of the treatment cells separately. The number of bootstrap samples drawn from each cell should be the same as the number in that cell in the original data, and these bootstrap samples associated with the individual cells can then be combined to find a replicate maximum likelihood estimate of the parameter values for the data set as a whole. If the data set is sufficiently large and if a sufficient number of bootstrap samples are taken, then the set of replicate estimates of a particular parameter will be approximately normally distributed. Testing whether a factor, continuous covariate, or interaction term has a significant effect in the model is then done by testing whether the mean of the set of a parameter's estimates is significantly different from zero (Ryan 1997). The bootstrap parameter estimate approach also allows an evaluation of each parameter's bias. Caution must be exercised when using the bootstrap to analyze data sets with limited sample sizes, however, because the data may inadequately reflect the shape of the presumed underlying negative binomial distribution.

Interpretation of numerical magnitude of parameter estimates (the β_j's) is straightforward if the indicator variables have been coded as described above, i.e., if a coding using zeros and ones has been used. Larger positive parameter values imply larger expected mean catches, while large negative values imply much reduced catches under the treatment associated with that parameter. Interpretation of the parameter values in the NBLIM model is the same.

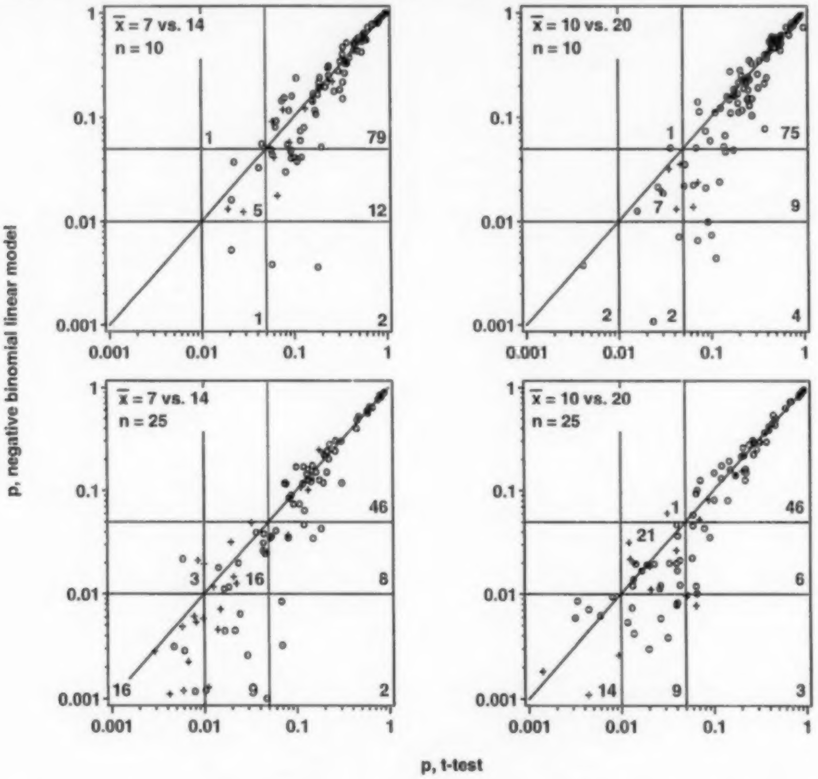
Simulations

Simulated data were generated to compare the negative binomial linear model approach described here with more classical methods. The simplest case is to assume that two sets of net catch sample data were collected under two different conditions and to compare them using both the t-test and the NBLIM. Sets of data were generated using pseudorandom number generators for the negative binomial distribution. In the first set of simulations, four groups of data were generated, with corresponding mean abundance values of 7, 14, 10, and 20. The value of 7 animals is the median abundance that Cyr et al. (1992) found in a literature survey of ichthyoplankton sampling. The data were generated so that the variance in each of the simulated data sets depended on the mean value, using the relationship of Cyr et al. (1992) for ichthyoplankton samples: $s^2 = 3.16\bar{x}^{1.74}$.

For each of the four specified mean (and variance) values, 100 sets of data, with 10 observations in each set, and another 100 sets of data, with 25 observations in each set, were generated. The sample size of 25 observations was chosen from examination of fig. 7 in Cyr et al. (1992). According to this graph, 25 net samples is roughly the number necessary to detect a difference (an effect size) between samples with mean abundances of 7 and 14 organisms or between samples with mean abundances of 10 and 20 organisms, with both statistical significance and statistical power at the 0.95 level. Most studies take fewer than 50 (= 25 × 2 treatments) samples, and so, sets of 10 samples were also generated.

A set of observations generated with a mean value of 7 was compared with data generated with a specified mean of 14, and a data set with a mean of 10 was compared to that with a mean of 20 using a t-test and the NBLIM. Each set of data did not necessarily have the specified mean value

Fig. 3. Comparison of the significance levels for the NBLIM analysis with a *t*-test, using untransformed data for the *t*-test. Means of the generated data being compared and the sample sizes used are given in each panel. Plus signs indicate data where Levene's test indicated unequal variances and Satterthwaite's correction was applied, and circles indicate data sets where Levene's test did not indicate unequal variances and no correction was applied. Horizontal and vertical reference lines are drawn at the 0.01 and 0.05 probability levels in each panel, and the diagonal line is the line of equal probabilities between the two tests. The number at the bottom right of a rectangular region is the number of points falling within that region.



because it was produced by a pseudorandom number algorithm. Although the paired data sets were generated so as to have unequal variances, in practice, this inequality of the variances would be unknown. To accommodate this, and emulate an investigator's approach to the data, the equality of the variances in the paired data sets was tested using Levene's test. If Levene's test indicated unequal variances, then Satterthwaite's correction for unequal variances was used; otherwise the uncorrected *t*-test was done. The probability level associated with the two-tailed *t*-test comparing the two groups was then plotted versus the probability associated with the NBLIM χ^2 test computed using the same pair of data sets. These tests were done using raw, untransformed data, and the results are presented in Fig. 3.

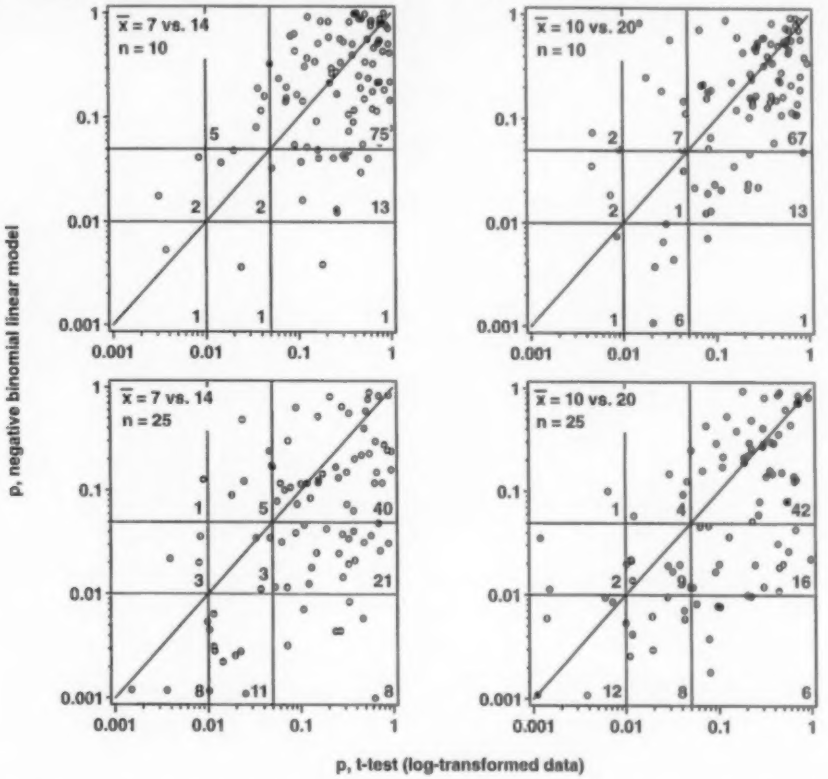
The logarithmic transformation is commonly used with net catch data. To emulate this practice, the generated sample data were multiplied by 100, 1 was then added to each value (to avoid taking the logarithm of zero), and the *t*-tests were repeated using the logarithmically (base 10) transformed data. Data were not transformed for the NBLIM analysis; hence, NBLIM compares the arithmetic mean of the two data sets, while the *t*-test of log-transformed data compares the geometric means. Results comparing *t*-tests of logarithmically transformed data to NBLIM are given in Fig. 4.

Groups of paired data sets were generated as before to examine the type I error performance of NBLIM, except that the specified means and variances in each pair of data sets were set to be equal to one another. Data sets with means of 10, 15, 20, and 25 were created, and for each of these mean values, sample sizes of 10, 20, 30, 40, and 50 were generated. One thousand pairs of data sets were generated for each of the 20 possible combinations of mean value and sample size, and the type I error rate at the 0.01 and 0.05 level for both the *t*-test and NBLIM approaches was determined and is available in Fig. 5.

Discussion

Using simulated data, the NBLIM does appear to provide results that are superior to those of the *t*-test for discerning a difference between the means of two treatments. Figure 3 shows that of the 400 test comparisons using untransformed data, there were 46 instances where the NBLIM detected a significant difference between two data sets at the $\alpha = 0.05$ (or less) level that the *t*-test did not detect whereas in only

Fig. 4. Same as Fig. 3, except comparing the NBLIM analysis with data that were logarithmically transformed for the *t*-test.



three cases did the *t*-test report a significant difference at the 0.05 level, while the NBLIM did not. Neither test reported a significant difference in 246 of the 400 comparisons.

Further, comparison of the probability levels associated with the two tests shows that the NBLIM frequently results in a lower probability level than the corresponding *t*-test. The diagonal lines in Fig. 3 are drawn to indicate equal probabilities between the two tests, and a majority of the data points lie below that diagonal line, indicating that the NBLIM results in a lower statistical probability level than the corresponding *t*-test with the same data.

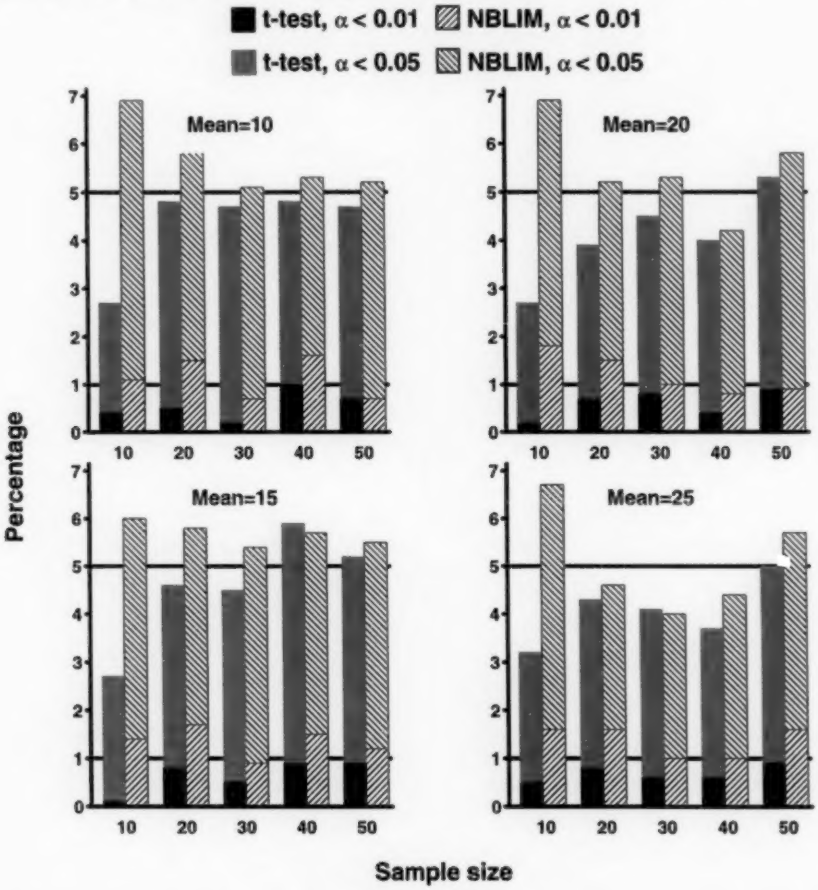
The same general results hold when the data are logarithmically transformed prior to the *t*-test, although the logarithmic transformation does result in a greater number of differences between the two treatments than were detected by the *t*-test. However, there appears to be much greater discrepancy between the *p* values obtained by the two methods, as indicated by the increased scatter of points in Fig. 4.

The type I error performance of the *t*-test is better than that of NBLIM (Fig. 5), although it appears acceptable for both methods in all cases. The poorest type I error performance occurred using NBLIM with small sample sizes (10 pairs of samples), but even in this case the error rate did not exceed 7%. These results confirm those presented by White and Bennetts (1996), who also found that their negative binomial models did not exhibit an excessive type I error rate.

The negative binomial is not a member of the exponential family of probability distributions when *k* is not specified a priori, but is instead estimated as a parameter. Because *k* is estimated as part of the model in the approach outlined here, the approach cannot strictly be termed a generalized linear model. It is also possible to use the approach with a fixed *k* specified in advance and to only estimate the parameters associated with covariates. The value of *k* associated with the fit to the Cyr et al. (1992) data presented in Fig. 2 is 0.922. Zweifel and Smith (1981) and Jahn (1987) provided estimates of *k* for ichthyoplankton samples that ranged from 0.045 to 1.114. Welch and Ishida (1993) provided substantially higher estimates of *k* in their analysis of salmon gillnet catches, indicating that these data more closely approach those of the Poisson distribution. Some authors have attributed an ecological interpretation to the dispersion parameter *k*, but Taylor et al. (1979) have criticized the use of the negative binomial *k* parameter as an ecological measure of organism aggregation. For the purposes of this paper, we do not assume any ecological significance of *k*. It should be noted that Clark and Perry (1989) and Piegorsch (1990) contended that maximum likelihood estimates of negative binomial parameters may be biased if sample sizes and parameter values are small. Bootstrap parameter estimates may allow estimation of this bias, but small sample sizes may not yield accurate parameter estimates when using the bootstrap as well.

Cameron and Trivedi (1986) also applied the negative binomial in analyses of count data. An important distinguishing aspect of the approach presented here is the allowance

Fig. 5. Results of simulating the type I error performance of the *t*-test and NBLIM approaches. Each bar is the percentage of 1000 tests that incorrectly reports a difference between two simulated data sets at either the $\alpha = 0.05$ or the $\alpha = 0.01$ level, when the two data sets were generated to have the same means. Data were generated having four mean values as indicated in each panel, and for each mean value, data sets of 10, 20, 30, 40, and 50 samples were generated.



for differing sample element sizes, which is usually an unavoidable aspect of sampling for aquatic organisms. If all sample element sizes are the same, then the method outlined here is identical to the Negbin II model of Cameron and Trivedi (1986).

Another approach to the analysis of net catch data would be to assume that the data follow a Poisson distribution and to use Poisson regression. Software for Poisson regression is more commonly available, and using this approach, one could estimate the parameters and then evaluate the results for overdispersion (Cameron and Trivedi 1990). It is then possible to use the estimate of overdispersion to adjust the standard errors of the model parameters. However, Poisson regression seems less desirable than the NBLIM if overdispersion is present, as is nearly always the case with samples of aquatic organisms. For this reason, no evaluation of Poisson regression compared with NBLIM was attempted in the work presented here; however, White and Bennetts (1996) concluded that Poisson regression performed poorly compared with their negative binomial models (and also when compared with ANOVA) when applied to data sets with overdispersion. McCleave et al. (1987) presented yet another approach in their efforts to relate the abundances of American eels (*Anguilla rostrata*) to depth of capture and tidal cycle. They presented a methodology for their data that assumed a multinomial distribution and used χ^2 goodness-of-fit hypothesis tests, but their method does not permit the incorporation of continuous covariates.

A comparative statistical analysis of the numbers of organisms caught is the crucial endpoint of many studies in aquatic ecology. It seems reasonable to assume that the better results demonstrated by the NBLIM simulations would also be obtained when applied to data sets with more than two treatments (mimicking a one-way ANOVA), factorial designs (mimicking a factorial ANOVA), data sets combining continuous and nominal exogenous variables (mimicking an ANCOVA), and data sets with continuous covariates only (mimicking a linear regression analysis). The NBLIM does not assume homogeneity of variances, and it incorporates the inherent nature of data that are restricted to contain only zero or positive integer values. Software for the NBLIM analysis using the SAS Interactive Matrix Language (SAS/IML) is available at http://www.lsu.edu/guests/jpower/nblim.html.

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Appendix

Obtaining the maximum likelihood solution requires finding the set of model parameters (values of κ_j and β_j) such that the gradient values ($\frac{\partial l}{\partial \kappa_j}$ and $\frac{\partial l}{\partial \beta_j}$) are zero. This is usually done numerically, with software that iteratively solves for the parameters. Although such solution strategies can utilize numerical approximations to the needed derivatives, greater efficiency can be obtained using analytical expressions; such algorithms also make use of a Hessian matrix with elements $\frac{\partial l^2}{\partial \kappa_j \partial \kappa_r}$, $\frac{\partial l^2}{\partial \kappa_j \partial \beta_r}$, and $\frac{\partial l^2}{\partial \beta_j \partial \beta_r}$. For the NBLIM approach using the canonical link, the gradient and Hessian matrix elements are given by

$$\frac{\partial l}{\partial \kappa_j} = \sum_{i=1}^{n} \left[x_{ij} \left(\psi(y_i + k_i) - \psi(k_i) - \ln\left(\frac{1 - [\exp(\eta_i)(1 - w_i)]}{1 - \exp(\eta_i)} \right) \right) \right]$$

$$\frac{\partial l}{\partial \beta_j} = \sum_{i=1}^{n} \left[\frac{x_{ij}[y_i(\exp(\eta_i) - 1) + k_i w_i \exp(\eta_i)]}{(\exp(\eta_i) - 1)(1 - [\exp(\eta_i)(1 - w_i)]} \right]$$

$$\frac{\partial l^2}{\partial \kappa_j \partial \kappa_r} = \sum_{i=1}^{n} [x_j x_r(\psi_1(y_i + k_i) - \psi_1(k_i))]$$

$$\frac{\partial l^2}{\partial \beta_j \partial \kappa_r} = \sum_{i=1}^{n} \left[\frac{x_j x_r w_i \exp(\eta_i)}{(\exp(\eta_i) - 1)(1 - [\exp(\eta_i)(1 - w_i)])} \right]$$

$$\frac{\partial l^2}{\partial \beta_j \partial \beta_r} = \sum_{i=1}^{n} \left[x_{ij} x_{ir} \exp(\eta_i) \frac{[(1 - w_i)[(y_i + k_i w_i) \exp(\eta_i)^2 - 2y_i \exp(\eta_i) + y_i] - k_i w_i]}{[(\exp(\eta_i) - 1)(1 - [\exp(\eta_i)(1 - w_i)])]^2} \right]$$

For the NBLIM approach using the logarithmic link, the gradient and Hessian elements are given by

$$\frac{\partial l}{\partial \kappa_j} = \sum_{i=1}^{n} \left[x_{ij} \left(\psi(y_i + k_i) - \psi(k_i) + \left(\frac{w_i \exp(\eta_i) - y_i}{k_i + w_i \exp(\eta_i)} \right) + \ln\left(\frac{k_i}{k_i + w_i \exp(\eta_i)} \right) \right) \right]$$

$$\frac{\partial l}{\partial \beta_j} = \sum_{i=1}^{n} \left[\frac{x_j k_i (y_i - w_i \exp(\eta_i))}{k_i + w_i \exp(\eta_i)} \right]$$

$$\frac{\partial l^2}{\partial \kappa_j \partial \kappa_r} = \sum_{i=1}^{n} \left[x_{ij} x_{ir} \left(\frac{y_i k_i + (w_i \exp(\eta_i))^2}{k_i (k_i + w_i \exp(\eta_i))^2} + \psi_1(k_i + y_i) - \psi_1(k_i) \right) \right]$$

$$\frac{\partial l^2}{\partial \beta_j \partial k_r} = \sum_{i=1}^{n} \left[\frac{x_{ij} x_{ir} w_i \exp(\eta_i)(y_i - w_i \exp(\eta_i))}{(k_i + w_i \exp(\eta_i))^2} \right]$$

$$\frac{\partial l^2}{\partial \beta_j \partial \beta_r} = \sum_{i=1}^{n} \left[\frac{-x_{ij} x_{ir} w_i k_i \exp(\eta_i)(y_i + k_i)}{(k_i + w_i \exp(\eta_i))^2} \right]$$

In all of the above expressions the $k_{j,r}$ and $\eta_{j,r}$ are first calculated from the linear combinations of the covariates for the *i*th observation. $\psi(\cdot)$ and $\psi_1(\cdot)$ are the digamma and trigamma functions, respectively.

Effect of winter food deprivation on growth and sexual maturity of Atlantic salmon (*Salmo salar*) in seawater

J. Duston and R.L. Saunders

Abstract: By identifying individual Atlantic salmon (*Salmo salar*) smolts (age 1+, mean weight 95 g), a tank study with four treatments (n = 175 fish per tank) indicated that maturation as grilse is associated with changes in somatic growth over many months, from July of the first summer in seawater onwards. The grilse rate in both sexes was associated with a relatively high condition factor from July onwards, but was independent of fork length. Food deprivation in either early winter (November–December, 8.2–4.1°C), late winter (February–April, 0.7–4.0°C), or both early and late winter (double deprived) reduced the female grilse rate ($p < 0.01$) to 4, 7, and 2% versus 18% in the control, whereas male grilse rate was reduced by a lesser degree ($p > 0.05$), 43, 39, and 33% versus 44% in the control. Mean condition factor among both maturing and immature fish decreased by a similar degree due to food deprivation but from spring onwards increased by a faster rate among incipient grilse. In a large experiment in four sea cages (each n = 3885 fish) using similar food deprivation treatments, the reduction in grilse rate was statistically significant in both sexes: among males, 28–36 versus 43% in the control; among females, 3–4 versus 6% in the control.

Résumé : Dans une étude en bassins avec quatre traitements (n = 175 poissons par bassin) dans laquelle des smolts de saumon atlantique (*Salmo salar*) (âge 1+, poids moyen de 95 g) étaient identifiés individuellement, on a observé que la maturation précoce (madeleineaux) était associée avec des changements dans la croissance somatique sur de nombreux mois, à partir du mois de juillet du premier été en eau de mer. Le taux d'apparition des madeleineaux chez les deux sexes était associé à un coefficient de condition relativement élevé à partir de juillet, mais était indépendant de la longueur à la fourche. La privation de nourriture au début de l'hiver (novembre à décembre, 8,2 à 4,1°C), à la fin de l'hiver (février à avril, 0,7 à 4,0°C) ou durant ces deux périodes (double privation) a réduit le taux d'apparition des madeleineaux femelles ($p < 0{,}01$) à 4, 7, et 2% contre 18% chez les témoins considérant que le taux d'apparition des madeleineaux mâles a été réduit par un peu de degré ($p > 0{,}05$), 43, 39 et 33% contre 44% chez les témoins. Le coefficient de condition moyen parmi tous les deux mûrissant et non mûrs les poissons a diminué par un degré semblable dû à la privation de nourriture mais à partir du printemps a agumenté à une vitesse plus rapide parmi les madeleineaux naissants. Dans une expérience de grande envergure effectuée dans quatre cages marines (n = 3 885 poissons par cage) utilisant les mêmes traitements de privation de nourriture, la réduction du taux d'apparition des madeleineaux a été statistiquement significative chez les deux sexes : dans le cas des mâles, il a été de 28 à 36 contre 43% chez les témoins, et dans le cas des femelles, de 3 à 4 contre 6% chez les témoins.

[Traduit par la Rédaction]

Introduction

Atlantic salmon (*Salmo salar*), after migrating to sea as smolts, mature sexually either after one sea winter (grilse) or after two or more sea winters. The importance of genotype on sea age at maturity is established (Gjerde 1984), but the interaction with environment, particularly the effect of seasonal growth on maturity, remains poorly understood. Thorpe (1986) proposed that the physiological decision to mature is dependent on some measure of either the rate of storage or turnover of surplus energy exceeding a genetically fixed threshold at a certain time of year. Exactly when this critical "decision period" occurs and its duration are uncertain. Some studies have associated sea age at maturity with growth in freshwater and body size at smolting (Ritter et al. 1986; Skilbrei 1989). Among smolts, Chadwick et al. (1987) found that the ovaries of high-grilse stocks were more developed compared with multi-sea-winter stocks. By comparison, Friedland and Haas (1996) correlated good growth during the first summer in seawater as postsmolts with an increased grilse rate. Thorpe et al. (1990) concluded that late winter in seawater was a critical period, since the incidence of grilse of a Scottish stock was reduced by food deprivation during this time. Later still, maturation as grilse has been associated with relatively high somatic growth during spring – early summer (Hunt et al. 1982; Herbinger 1987; Skilbrei 1989; McLay et al. 1992). This spring increase in growth

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may be a consequence of the commencement of gonadal maturation rather than a cause (Hunt et al. 1982), yet subpopulations of Atlantic salmon may make the decision to mature as grilse very late, perhaps within 4–5 months prior to spawning (Youngson et al. 1988).

To determine if a specific period of winter growth in seawater affected the grilse rate, we identified individual Atlantic salmon smolts at age 1+ and then subjected four treatments groups in tanks to periods of food deprivation during either early or late winter or both periods. Somatic growth through to maturity at age 2+ was also monitored. A second large-scale experiment in sea cages, without the detailed growth data, served as a replicate.

Materials and methods

Laboratory experiment

Experimental Atlantic salmon were a mixture of up to 40 families of a high-grilse strain derived from seven rivers in New Brunswick (O'Flynn et al. 1993). Potential smolts (>14 cm fork length in February, age 1+) reared from eggs at the Atlantic Salmon Federation, St. Andrews, N.B., were transported to the Biological Station in February 1991. The fish were reared in a single tank with a flow-through supply of freshwater. In late April, when smolting was complete, the water supply was switched from freshwater to seawater (29–31‰). On April 29, fish were randomly distributed among four cylindrical tanks (1.8 m in diameter, 3.0-m^3 volume). Individuals were identified with a PIT tag (Prentice et al. 1990) inserted into the interperitoneal cavity via a small incision in the ventral body wall. Fish were hand-fed to satiation four times per day with a commercial moist diet (Connors Bros., Limited) except during food deprivation treatments. Food deprivation commenced November 17 with four experimental treatments, initially n = 175 fish per treatment: (*i*) early deprived (early winter, November 17 – December 21), (*ii*) late deprived (late winter, February 16 – April 29), (*iii*) double deprived (early and late winter), and (*iv*) control (fed to satiation throughout). Fish in the early-deprived treatment were not fed during alternate weeks between November 17 and December 21 (November 17–23, December 1–7, December 14–21). Fish in the late-deprived regime were not fed during February 16–22 (7 days), March 1–14 (14 days), March 26 – April 4 (10 days), and April 12–29 (18 days). Seawater temperature was ambient (monthly mean range 1.1°C in February to 12.2°C in September). Photoperiod was simulated natural (latitude 45°N) provided by fluorescent lights with no twilight period. At 1- to 2-month intervals, between November 1991 and November 1992, individuals were removed at random from each group until six males and six females were obtained. A total of 382 fish were removed from the experiment. Recorded from each fish were fork length (nearest 0.1 cm), body weight (BW) (nearest 0.1 g), sex, and gonad weight (GW) (nearest 0.1 g). Gonadosomatic index (GSI) was calculated as GSI = GW·100/BW. In addition, at 1- to 2-month intervals, all remaining fish in each tank were anesthetised and body weight, fork length, and PIT tag code recorded. Eleven males (about 3% of males) that sexually matured at age 1+ as postsmolts in autumn were removed from the experiment. The experiment ended on November 10, 1992, when all remaining fish in the four groups were killed and sex was determined by visual inspection of the gonads. The incidence of sexual maturity was calculated from those fish remaining at the end of the experiment plus those fish sampled from late April onwards, when gonadal development allowed maturing fish to be easily identified. Analysis of somatic growth over the 1.5-year study was performed on only those fish alive at the end of the experiment (n = 66–77 per treatment).

Sea cage experiment

Seventeen thousand smolts of the same stock (see above) raised at the Atlantic Salmon Federation were transferred in May 1991 to a single sea cage (12 × 12 × 6 m) at a Connors Bros., Limited site (Fairhaven, Deer Island, N.B.). In October 1991 the fish were randomly distributed by hand among four cages, n = 3885 fish per cage. Food deprivation treatments were similar to the tank experiment, but the timing differed. In the early-winter deprivation treatment (November–January), fish were not fed during weeks 47, 49, and 51 of 1991 and week 1 of 1992. In the late-winter food deprivation treatment (February–May 1992), fish were not fed during weeks 8, 10, 11,13, 14, 16, 17, 19, and 20. Apart from these periods, fish were fed to satiation a moist diet via an automatic delivery system (Oppdrett). Incidence of grilse was determined in September 1992 by examination of gonads at processing following harvest.

Statistical analyses

Means were compared either by analysis of variance incorporating Tukey's multiple range test or by pairwise Student's t test when comparing mature versus immature fish within each treatment and sex. Specific growth rate = (ln WT_2 – ln WT_1)·100/($t_2 - t_1$) where $t_2 - t_1$ is the number of days between measurements and WT_2 and WT_1 are the individual body weights on those dates. Condition factor = BW·100/FL^3 was a satisfactory index of "fatness," as most groups compared had a similar mean fork length (Cone 1989). Condition factor and GSI data were arcsine transformed prior to analysis. Incidence of sexual maturation was compared by G test based on the intrinsic null hypothesis (a – 2 degrees of freedom) that treatment had no significant effect on the incidence of maturation (Sokal and Rohlf 1969). Where the null hypothesis was rejected, pairwise comparisons were achieved by calculating the 95% confidence intervals for each frequency involving use of the Bonferroni Z statistic (Neu et al. 1974). A significance level of p = 0.05 was applied in all tests.

Results

Laboratory experiment

Summarising the overall growth (n = 66–77 per treatment), the mean weight of postsmolts increased from about 90 g in June to 400–450 g on November 14. Mean condition factor increased from between 1.03 and 1.07 in June to between 1.17 and 1.20 in November, with no significant differences between treatments. Food deprivation in early winter (November 17 – December 21) reduced the mean (SE) specific growth rate (November 14 – January 5) to 0.1%·day^{-1} (0.01) in both the early- and double-deprived treatments compared with 0.4 (0.13) and 0.3%·day^{-1} (0.02) in the control and late-deprived treatments. Mean condition factor of both the early- and double-deprived treatments decreased significantly to 1.12–1.13 at the end of the early-winter deprivation period and then remained at this level through to mid-February. By comparison, in both treatment groups fed to satiation in early winter, their mean condition factor exhibited no significant change from November to February, remaining between 1.16 and 1.20. The second period of food deprivation (February 16 – April 29) resulted in mean condition factor of the late- and double-deprived treatments decreasing to 1.06 (0.010) and 1.05 (0.008), respectively ($p > 0.05$), on April 29, both significantly lower than the early-deprived treatment, 1.10 (0.010), and all three significantly lower than the control, 1.16 (0.011). Mean weight of the double- and late-deprived groups increased by <20 g between February

12 and April 29 compared with gains of 75–80 g in the two groups fed to satiation. On April 29 the mean weight of the control, 667 g (18.4), was highest ($p < 0.05$) and that of the early-deprived treatment, 581 g (19.9), was significantly higher than that of both the late- and double-deprived treatments, 509 (18.9) and 481 g (15.5), respectively. From May onwards, all groups exhibited rapid growth driven by the seasonal increase in temperature. On November 12, the overall mean weight of the control, 1883 g (61.6), was the highest ($p < 0.05$). All other groups did not differ significantly, ranging from 1665 (60.4) to 1794 g (58.9). Final mean condition factor showed no significant differences among treatments, range 1.35 (0.015) to 1.38 (0.017).

The effect of treatment on the incidence of grilse in the tank experiment was significant among females ($G = 10.53$, $p \leq 0.01$, 2 df) but not significant among males ($G = 1.20$, $p > 0.05$), based on fish judged mature at the end of the study plus those lethally sampled within the final 6 months (Table 1). Among females, the grilse rates among the three food deprivation treatments were not significantly different (1–4 ± 2–3%, 95% confidence intervals), but all were significantly lower than those of the control (18 ± 6%). Among males, the incidence of grilse ranged from 32.8% in the double-deprived treatment to 43.8% in the control, yet the differences were not statistically significant.

Comparisons of somatic growth between immature and maturing fish within each sex and treatment revealed marked differences in condition factor but only minor differences in mean fork length until completion of maturation. Among males, the mean condition factor of future grilse was significantly higher than that of immatures from the first summer in seawater onwards in all treatments except the double-deprived treatment (Fig. 1). Food deprivation in both early and late winter resulted in decreases in mean condition factor of similar magnitude among both maturing and immature males. Loss of body weight due to food deprivation was minor; decreases in mean weight between measurements occurred only between late March and late April, and then <10 g among fish of 470–580 g mean weight. The decreases in condition factor were due to food deprivation reducing growth rate in terms of weight to a greater extent than growth rate in terms of fork length. Among females, a comparison of growth between maturing and immature fish was possible only in the control (Fig. 2). In the three food deprivation treatments, only two of 101 surviving females were mature at the end of the study. Within the control treatment, mean condition factor of females that matured at age 2+ remained significantly higher than that of immature females from July 1991, over 1 year prior to completion of maturation, to August 1992.

Comparisons of mean fork length between immature and maturing fish up to the end of April revealed significant differences only among males in the control where the maturing males were significantly smaller than the immature males (Fig. 1). Almost all other pairwise comparisons of either mean fork length or body weight between future grilse and immature fish in either sex revealed no statistically significant differences. The only two exceptions were incipient male grilse that had a significantly greater mean body weight than immature males in the early- and late-deprived treatments in July and October 1991, respectively.

In spring, all fish exhibited a rapid increase in growth rate and condition factor, but the rate of increase was greatest in sexually maturing fish. Pooling data from all treatments, the change in condition factor between April and June was a good indicator of maturation (Fig. 3). All 11 males that exhibited an increase in condition factor greater than 0.2 during this period matured in autumn whereas only 22% (11/51) matured that showed an increase in condition factor of between 0.00 and 0.04 during April–June. Among females, 100% of fish that spawned exhibited an increase in condition of greater than 0.10 between April and June whereas all those with an increase in condition of less than 0.09 remained immature. Condition factor of both maturing males and females peaked around August and then decreased significantly in association with the completion of sexual maturity. By November, in all treatments, the mean condition and weight of the mature fish were significantly lower than those of the immatures, both among males (about 1.4 versus 1.8 kg) and among females (about 1.5 versus 2.1 kg).

GSI was not affected by the treatments, so data were pooled. Among males, mean (SE) GSI increased from 0.05% (0.01) in November to 0.08% (0.01) in April ($p < 0.05$). From April onwards, the GSI of maturing and immature males diverged. The mean GSI of maturing males in June was 0.5% (0.05) compared with 0.08% (0.01) in immatures and in November was 7.6% (1.42) compared with 0.12% (0.02) in the immatures. Among females, mean GSI increased significantly overwinter from 0.15% (0.01) in November to 0.2% (0.01) in March and then increased to 0.4% in June 1992 and up to 20–25% at spawning. Mean GSI of immature females remained less than 0.2% through September 1992 and then increased significantly to 0.25% (0.01) in November.

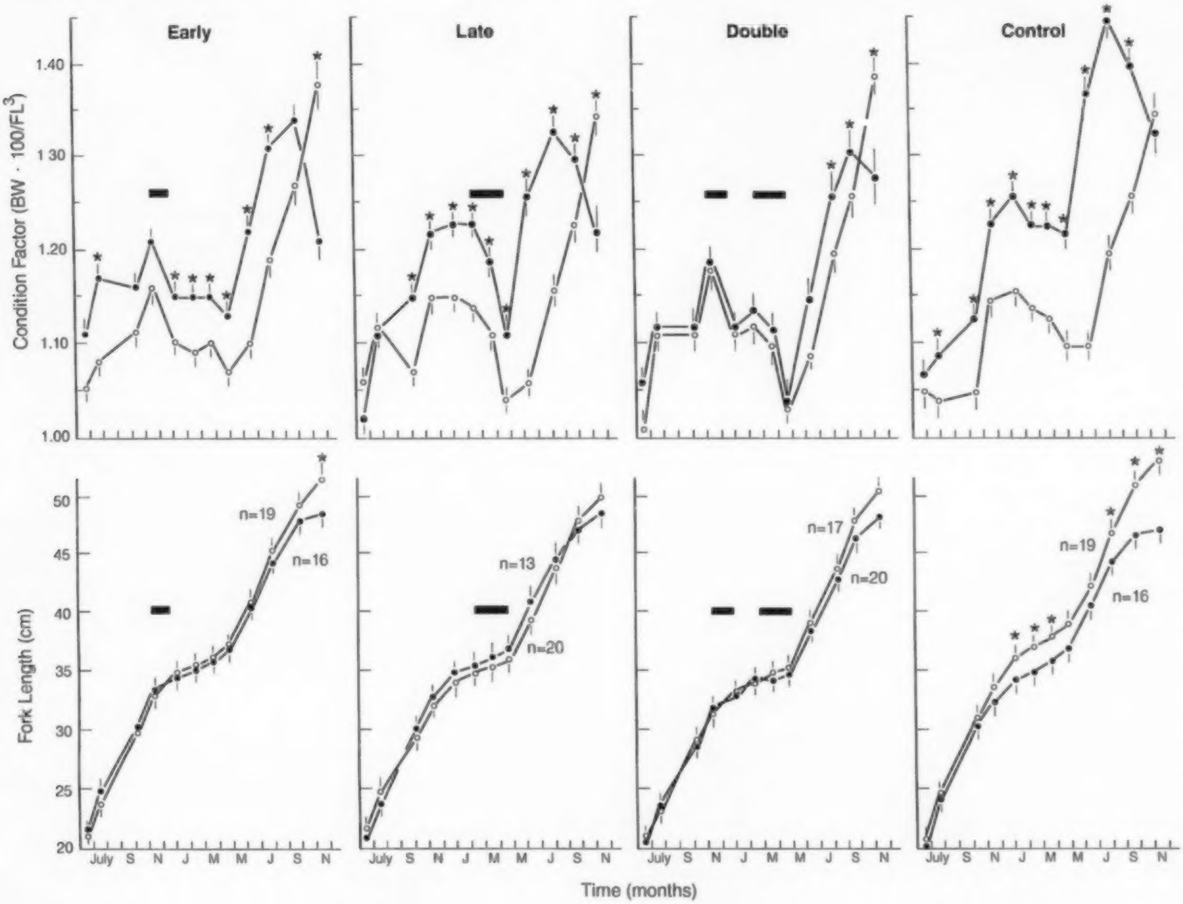
Sea cage experiment

All 13 343 fish were harvested in September 1992, range 3030–3516 per cage; 50.2% were females. In both sexes, the incidence of grilse was significantly ($p < 0.001$) reduced by food deprivation (Table 1). Among males, the incidence of grilse between the early- and late-deprived treatments was not significantly different (34. 6 ± 1.4 versus 36.4 ± 1.5%) whereas all other pairwise comparisons were statistically significant. Similarly, among females the grilse rate was significantly different in all pairwise comparisons except the early- and late-winter food deprivation treatments (both 3.9 ± 0.6%). Overall mean weight at harvest of the control, early-, late-, and double-deprived treatments was similar, 2.34, 2.32, 2.36, and 2.34 kg, respectively. Statistical comparisons of these means are not valid, since the cages were harvested at different dates in September, ranging from the first week (control) to the last week (double-deprived treatment).

Discussion

The new finding that maturation as grilse was associated with a relatively high condition factor from the early post-smolt stage onward suggests that the "decision" to mature is influenced by "fatness" over 1 year prior to spawning. Furthermore, that the incidence of grilse can be reduced by poor growth at any period through winter indicates that the out-

Fig. 1. Comparison of growth in seawater between male Atlantic salmon sexually mature in November at age 2+ (solid circles) and immature (open circles). Shown are mean (±SE) condition factor and fork length. Bold horizontal bars indicate the periods of food deprivation in either early winter (November 17 – December 21), late winter (February 16 – April 29), or both early and late winter (double deprived) or fed to satiation throughout (control). Within each treatment, asterisks indicate significant differences between means (Student's *t* test).



come of the decision to mature is influenced by somatic growth over many months.

The divergence in condition factor during the first summer in seawater between maturing and immature fish, double-deprived treatment excepted (Figs. 1 and 2), extends the conclusions of Friedland and Haas (1996) that good growth as early postsmolts is linked to maturation after one sea winter. Condition factor is significantly correlated with body fat levels, in parr at least (Herbinger and Friars 1991), which have been implicated in the decision to mature in Atlantic salmon (Thurow 1966; Rowe et al. 1991). Whether this early change in condition factor is a cause or an effect of maturation is uncertain, but the results do indicate that age at maturity is influenced by growth much earlier in life than the late-winter period emphasised by some studies (Rowe and Thorpe 1990; Thorpe et al. 1990; Berglund 1995). Similar to our data, Bohlin et al. (1994) concluded that maturation in male brown trout (*Salmo trutta*) was associated with relatively high condition factor over 1 year prior to completion of the event. Further evidence that reproductive investment in sea-run Atlantic salmon is affected by somatic growth relatively early in life is restricted to smolt data and offers no clear consensus. Chadwick et al. (1987) concluded that genotype, rather than effects of environment on freshwater growth, was the dominant factor controlling the incidence of grilse, since ovarian development among smolts (but not smolt size) correlated with sea age at maturity. Jonsson et al. (1996) also found that smolt size did not affect the incidence of grilse but did affect reproductive investment in terms of fecundity and egg size. In contrast, other studies detected a significant positive relationship between smolt size and the incidence of grilse (Ritter et al. 1986; Skilbrei 1989).

Among females in our study, their greater sensitivity to food deprivation and lower incidence of maturity compared with males have been observed previously (Thorpe et al. 1990; Silverstein and Shimma 1994) and can be attributed to the greater energetic cost of female maturation. The major decrease in female grilse rate by all three deprivation treat-

Fig. 2. Comparison of growth in seawater between female Atlantic salmon sexually mature in November at age 2+ (solid circles, *n* = 9, control group only) and those remaining immature in the four treatment groups (open symbols, *n* = 31–35 per group). Shown are mean (±SE) condition factor and fork length. Four treatments groups were subjected to food deprivation in either early winter (November 17 – December 21, open squares), late winter (February 16 – April 29, inverted triangles), or both early and late winter (triangles) or fed to satiation throughout (control, open circles). Bold horizontal bars indicate the periods of food deprivation.

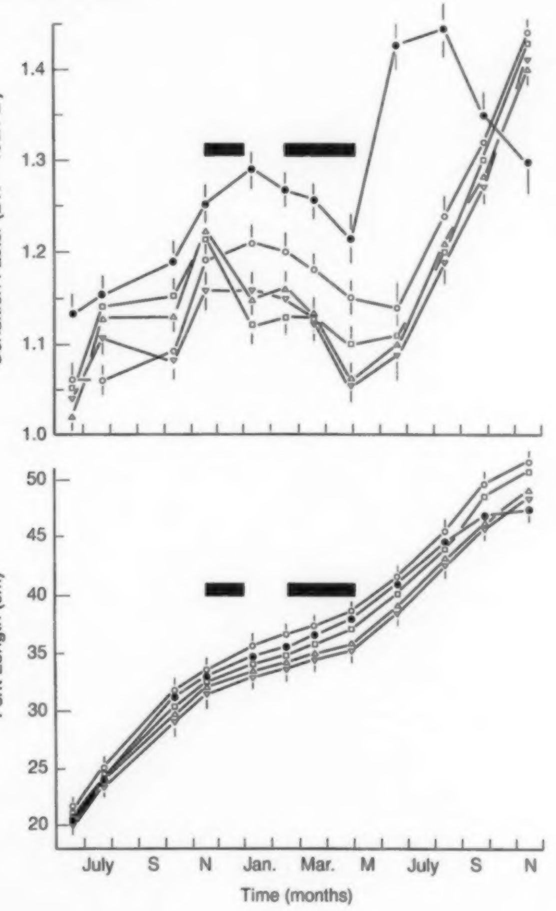


Fig. 3. Incidence of sexual maturation in autumn at age 2+ among male (open circles) and female (solid circles) Atlantic salmon in relation to the increase in condition factor between April 29 and June 16 measurements. Data are pooled from all four treatments. Numbers next to coordinates indicate *n*, the number of individuals in each category.

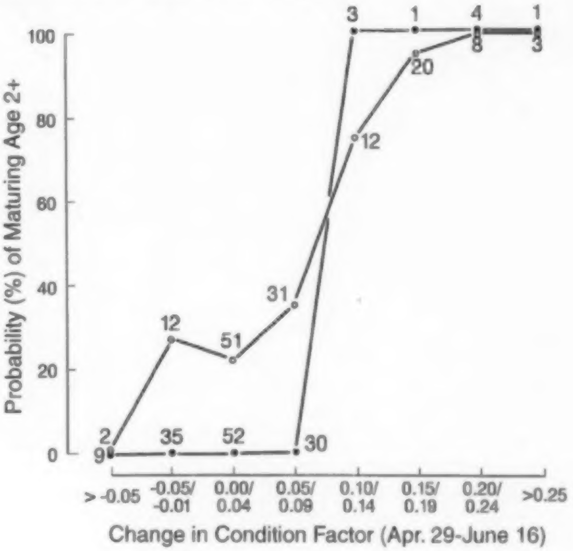


Table 1. Effect of food deprivation in early and/or late winter on the incidence (%) of grilse in both tank and sea-cage experiments.

		Treatment			
		Early deprived	Late deprived	Double deprived	Control
Males					
Tank	*n*	61	52	61	64
	%	42.6	38.5	32.8	43.8
Sea cage	*n*	1612	1768	1468	1804
	%	34.6	36.4	27.8	42.6
Females					
Tank	*n*	62	62	54	61
	%	4.8	6.5	1.9	18.0
Sea cage	*n*	1605	1812	1562	1712
	%	3.9	3.9	2.9	5.9

Note: *n* is the total number in each population.

ments in tanks indicates that this sex must maintain a consistent condition throughout winter to satisfy the genetically set requirements to mature as grilse. Among males, data from all but the double-deprived treatment indicated that fatness entering winter is the most critical factor affecting the decision to mature, since subsequent winter food deprivation reduced mean condition factor in both maturing and immature fish by a similar degree and resulted in only moderate reductions in the grilse rate (Fig. 1). We question whether there is any causal relationship between condition factor in late winter and maturation in males, since, strikingly, the mean condition factor of maturing males in the double-deprived group during this period was actually lower than that of immature males in the control treatment (Fig. 1). In Scotland, Thorpe et al. (1990) found that food deprivation between February and April was twice as effective as similar treatments earlier in winter in reducing the female grilse rate and was the only period appearing to reduce the male grilse rate. The lack of effect of early-winter food deprivation compared with our results might be attributable to the higher winter seawater temperatures around Scotland than in the Bay of Fundy (7 versus 1°C) enabling previously starved fish to feed, recover condition, and remain "on track" to mature as grilse. Saunders et al. (1988) concluded that winter temperature has a major influence on sea age at maturity, since the grilse rate was greatly reduced among fish reared in sea cages in the Bay of Fundy at 1°C overwinter compared with wild fish of the same stock that mature exclusively as grilse and may overwinter in seawater warmer than 4°C (Reddin and Shearer 1987). Why a higher female grilse rate occurred in tanks compared with sea cages is uncertain (Table 1). Since temperature at the two sites was similar, possibly careful hand-feeding as opposed to automatic feeders was a factor.

The best predictor of maturation in both sexes was the increase in condition factor between April and June (Fig. 3). We believe that this increase in condition factor was an effect rather than a cause of maturation, since it occurred concomitantly with increases in GSI and may be attributed to the known anabolic effect of sex steroids on somatic growth (Hunt et al. 1982). It would be interesting to determine whether food deprivation during this late period reduces the incidence of grilse or merely reduces reproductive investment by individuals, as observed in other studies (Hislop et al. 1978; Bromage and Cumaranatunga 1988). The similar growth in terms of mean fork length between maturing and immature fish until close to completion of maturity confirms previous studies on Atlantic salmon in seawater (Hunt et al. 1982; Youngson et al. 1988). The compensatory growth following a return to full ration indicates that losses in meat production may be acceptably low for food deprivation to be used in aquaculture as a tool to reduce the problem of early maturation (Asknes et al. 1986). In summary, this study showed that maturation as grilse is linked to changes in growth from the early postsmolt stage through the first winter in seawater. The possibility that this reproductive investment can be influenced by the effect of environment on growth even earlier in life, during the freshwater phase, deserves closer examination.

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Evaluating uncertainty in harvest control law catches using Bayesian Markov chain Monte Carlo virtual population analysis with adaptive rejection sampling and including structural uncertainty

K.R. Patterson

Abstract: A new method is developed for calculating Bayes posterior distributions of future catches that conform to a specified harvest control law while incorporating uncertainty in biological reference points, natural mortality, and some aspects of model structure in addition to the usual stochastic noise. A Markov chain Monte Carlo approach is used to calculate Bayesian posterior distributions for critical parameters of a Norwegian spring-spawning herring (*Clupea harengus*) stock assessment using an assessment model that incorporates catch-at-age, survey, and tag release and recapture observations. Exceptionally, the approach allows prior uncertainty in model structure (e.g., whether survey observation errors should be treated as normal, lognormal, or gamma variates; whether Ricker or Beverton–Holt forms are used to model recruitment). This modelling approach is a useful tool that allows management advice to be provided that takes into account uncertainty in model structures and in some parameters that, by conventional methods, need to be specified as arbitrary "best" choices. The method is also used to quantify uncertainty in some biological reference points and to calculate a probability distribution for a future catch with respect to a specified harvest control law. This has the advantage of a consistent treatment of uncertainty throughout the process of stock modelling, reference point estimation, and concomitant catch forecasting.

Résumé : Nous mettons au point une nouvelle méthode de calcul des distributions bayésiennes a posteriori des prises futures qui se conforment à une loi précise de contrôle des prélèvements tout en intégrant l'incertitude dans les points de référence biologiques, la mortalité naturelle et certains aspects de la structure du modèle, en plus du bruit stochastique habituel. Une approche chaîne de Markov Monte Carlo sert à calculer les distributions bayésiennes a posteriori pour les paramètres critiques de l'évaluation d'un stock de harengs (*Clupea harengus*) géniteurs de printemps en Norvège à l'aide d'un modèle d'évaluation qui intègre les captures selon l'âge, les données de relevés scientifiques et les observations de lâcher et de recapture de poissons marqués. Exceptionnellement, la méthode permet une incertitude a priori dans la structure du modèle (p.ex., les erreurs d'observation dans les relevés peuvent être traitées comme des variables aléatoires normales, lognormales ou gamma; on peut se servir de la formule de Ricker ou de celle de Beverton–Holt pour modéliser le recrutement). Cette méthode de modélisation est un outil précieux qui permet de fournir des avis de gestion tenant compte de l'incertitude dans les structures des modèles et dans certains paramètres qui, selon les méthodes conventionnelles, doivent être désignés comme des « meilleurs choix » arbitraires. La méthode sert aussi à quantifier l'incertitude dans certains points de référence biologiques et à calculer une distribution de probabilité d'une capture à venir par rapport à une loi précise de contrôle des prélèvements. Elle présente l'avantage de permettre de traiter de façon uniforme l'incertitude tout au long du processus de modélisation d'un stock, d'estimation des points de référence et de prévision concomitante des prises.

[Traduit par la Rédaction]

Introduction

Current discussion on the provision of fishery management advice is centred on the comparison of some measure of stock dynamics (e.g., percentiles of the distribution of spawning stock biomass or fishing mortality F) with some target or limit measure derived from historical parameters estimated from the stock (F_{med}, F_{msy}, G_{loss}, etc.) (Anonymous 1996*c*, 1997*a*; Cook 1998). A method is proposed here that allows estimation of uncertainty in both the stock assessment and in the reference points so that advice can be given to management in the form of expected values and probability statements about the levels of catch that will conform to a given harvest control law. It can be used to address problems of providing advice in cases where F is low and close to currently assumed, but very uncertain, levels of natural mortality M.

The method can be used to address questions of structural uncertainty, such as which error distribution model best represents the spread of observations or which stock–

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Table 1. Summary of some assumed values of natural mortality and of estimated fishing mortality for some major fish stocks in the Northeast Atlantic.

Stock	Adult natural mortality	Fishing mortality in 1994	Estimate of $F_{0.1}$
North Sea cod (*Gadus morhua*)	0.35 (age-1) to 0.2	0.94	0.15
North Sea haddock (*Melanogrammus aeglefinus*)	1.65 (age-1) to 0.2	0.91	0.20
North Sea whiting (*Merlangius merlangus*)	0.95 (age-1) to 0.2	0.67	0.23
Norwegian spring-spawning herring (*Clupea harengus*)	0.15	0.26	0.15 (target)
North Sea herring (*Clupea harengus*)	1 (age-1) to 0.1	0.67	0.2 to 0.3 (proposed)
Western mackerel (*Scomber scombrus*)	0.15	0.30	0.15 to 0.2 (proposed)
Western horse mackerel (*Trachurus trachurus*)	0.15	0.18	
Herring in West Scotland (*Clupea harengus*)	1 (age-1) to 0.1	0.12	0.136

recruitment model best represents the historical relationship of recruitment and spawning stock size (SSB). It is fairly easily applicable as long as likelihood functions for the stock assessment model can be calculated, prior distributions for the unknown parameters can be specified, and if a formal harvest control law has been specified. Norwegian spring-spawning herring (*Clupea harengus*) is used for example calculations.

Uncertainty in *M*, survey error structure, stock–recruit model type, and biological reference points has been modelled using this approach. This is discussed below.

Many reference points are directly and strongly dependent on estimates of *M* (e.g., $F_{0.1}$, $F_{\max}$). For other reference points such as F_{med} or F_{loss}, the dependence on *M* may be reduced if they are only used in a comparative fashion (e.g., computing the probability that a current *F* may exceed the reference). However, even in such cases and where time series are short, there remains the strong possibility that misspecification of *M* may induce spurious time trends in estimates of historical population parameters, which could bias estimates of the reference points (Lapointe et al. 1989). In the current situation with most demersal stocks in the North Sea, this has usually been considered fairly unimportant, as the rate of *F* dominates the stock dynamics, exceeding the assumed rate of *M* by three to five times (Table 1). However, that is not necessarily always the case, particularly where stocks are managed conservatively. Pelagic fish stocks must be managed at quite low levels of *F* to avoid known problems with stock–recruit collapses and concentration effects. Among the main pelagic stocks assessed in the North Atlantic, current *F* rates range from below *M* to twice *M* (with the exception of North Sea herring, for which emergency conservation measures have been implemented; Anonymous 1997*b*).

Estimates of *F* made using virtual population analysis (VPA) are conditional on assumed values of *M*. The basis in data for values of *M* used in the assessment and advisory procedure is often weak, although improved estimates of predation-dependent *M* can be calculated using multispecies approaches. Arguably, uncertainty in *M* should routinely be included in cases where advice is to be given on the medium- or long-term development of a stock in comparison with some specified reference points.

Most fish stock assessments are calculated on the assumption of lognormality in survey errors, but this assumption may on occasion appear to be violated, and other models (e.g., gamma) may appear to be more appropriate. However, altering error model assumptions may result in large alterations in perceptions of stock size, and the basis in data for choosing one assumption over another may not be very strong. This is a source of uncertainty that is rarely considered but that is reasonably tractable under the present approach.

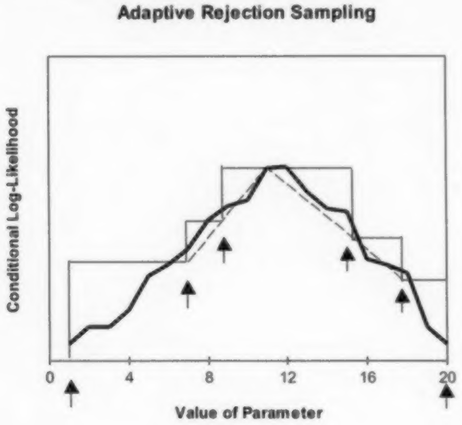
In a similar way, the appropriate form of stock–recruit model may have large consequences for perceptions of stock development in the medium term, but the choice of model may be weakly determined by data and strongly determined by subjective preferences. Again, this type of uncertainty may be modelled by the procedure suggested here.

Most fishery reference points are taken as deterministic values, and the uncertainty attached to reference point estimates has only recently come under consideration (Anonymous 1997*d*), although an exception is the G_{loss} reference point, where the approach has been to evaluate sustainability of the fishery in terms of the probability that a fishery is above or below the G_{loss} sustainability criterion (Cook 1998). Reference points will typically depend most strongly on assumed values of *M*, but also on an estimated selection pattern and growth and maturity parameters.

Most of the values of the reference points more frequently used for management purposes and listed in Anonymous (1997*a*) are *M* dependent. Also, most target reference points for management indicate low long-term levels of *F*, and these are often quite close to, or below, *M*. In some cases, this is stated explicitly, e.g., $F = 2/3M$ and $F = M$ have been proposed as target reference points (Patterson 1992; Walters and Maguire 1996). These reference points obviously depend directly on the assumed value of *M*, while others depend indirectly. It is not immediately clear how uncertainty in values of *M* introduces uncertainty in both the estimation of current stock size and the calculation of reference points or how this affects short-term advice based on a certain harvest control law.

In cases where the dynamics of a stock are at low levels of *F*, there is therefore a significant problem in the provision of advice, both in the objective estimation of current stock size and the determination of appropriate target reference points. In the North Sea and adjacent areas, only some pelagic stocks are presently in such an area of stock dynamics. However, in principle the application of the precautionary approach (Anonymous 1997*d*) would lead other stocks into this area, which is problematic for parameter estimation. The approach suggested here is intended as an objective and comprehensive procedure to incorporating these sources of uncertainty and their interdependencies when assessing a fish

Fig. 1. Construction of upper envelope function for adaptive rejection sampling. Thick line, conditional probability distribution of unknown form; thin line, constructed upper envelope function; broken line, construction line. Arrows show the points at which the function was evaluated. New points are added and the envelope readjusted until a sample has been taken from the conditional distribution, which typically takes five to eight function evaluations.



stock and providing advice or short- to medium-term exploitation.

Methods

Conventional Bayesian analysis relies on an evaluation of the posterior probability $P(\boldsymbol{\Theta}|\mathbf{X})$ of a certain set of assumptions $\boldsymbol{\Theta}$, given prior belief $P(\boldsymbol{\Theta})$ about those assumptions, a set of data $\mathbf{X}$, and a likelihood function allowing the evaluation of $P(\mathbf{X}|\boldsymbol{\Theta})$. The conditional probability can be expressed as

$$(1) \qquad P(\boldsymbol{\Theta}|\mathbf{X}) = \frac{P(\mathbf{X}|\boldsymbol{\Theta})P(\boldsymbol{\Theta})}{\int P(\mathbf{X}|\boldsymbol{\Theta})P(\boldsymbol{\Theta})\mathrm{d}\boldsymbol{\Theta}}.$$

Conventionally, the $\boldsymbol{\Theta}$ are usually a vector of input parameters to a model **M**, which is assumed to be correct and upon which the likelihood function is predicated. It is also possible to treat the entire model structure **M** as being an additional parameter and to integrate over uncertainty both in the model structure **M** and in the model parameters. In this case, each $\boldsymbol{\Theta}$ has a meaning that is specific to each **M.** This method, suggested by Draper (1995), differs from the usual approach of using available data to choose a "most appropriate" model and then proceeding on the assumption that the chosen model is correct. The method also provides a posterior perception of the likelihoods of each structural model being a true representation of the data structure.

The evaluation of posterior probabilities proceeds exactly analogously:

$$(2) \qquad P(\boldsymbol{\Theta},\mathbf{M}|\mathbf{X}) = \frac{P(\mathbf{X}|\boldsymbol{\Theta},\mathbf{M})P(\boldsymbol{\Theta}|\mathbf{M})P(\mathbf{M})}{\int P(\mathbf{X}|\boldsymbol{\Theta},\mathbf{M})P(\boldsymbol{\Theta},\mathbf{M})P(\mathbf{M})\mathrm{d}\boldsymbol{\Theta}\mathrm{d}\mathbf{M}}.$$

It is obviously possible by this approach to evaluate a posterior probability distribution for any quantity of management interest (such as a catch forecast or medium-term projection) that can be expressed in terms of $\boldsymbol{\Theta}$ and **M**. Such a distribution can be constructed for any reasonable range of alternative structural model components in **M**, assuming that for each model component, a likelihood term $P(\mathbf{X}|\boldsymbol{\Theta},\mathbf{M})$ can be calculated.

Norwegian spring-spawning herring

This stock is chosen as suitable for a case study because (*i*) it is fished close to assumed values of *M*, and there exists tagging information that should be informative about this parameter, (*ii*) the appropriate form of stock–recruit relationship is undecided but important in planning future exploitation, (*iii*) the assessment is sensitive to the choice of assumptions about the appropriate error distribution model for the surveys, and (*iv*) a disease-induced fish kill is believed to have had a significant impact on the stock in recent years, but there are few observations. In the case of the Norwegian spring-spawning herring, uncertainty in model structure has led to poor consistency in advice. For example, Anonymous (1996*b*) considered alternative structural assumptions about the error distributions of the acoustic surveys used to measure the stock size. On the basis of residual plots, they noted that an assumption of lognormal errors conformed better to the observations than an assumption of normal errors, and so adopted the assumption of lognormality for assessment purposes. However, this change resulted in a change in the perception of the size of the 1983 year-class in the beginning of 1995 from 1.8 to 3.5 billion individuals. As there are few observations from the surveys, the basis for making a choice of "best" assessment model by comparing error distributions is not very strong. The model uncertainty approach described here can provide a way in which management advice can be supplied without forcing a choice of best assessment model and without losing a perception of the uncertainty due to lack of knowledge about the true structural model. Additional problematic features of this stock assessment are that *M* is unknown and that a recent outbreak of a disease-induced mortality is thought to have had a significant impact on the stock, but this effect has proven hard to quantify.

Problems of appropriate parameterization and perceptions of risk are addressed by examining posterior distributions for appropriately structured models. By this means, the feasibility of estimating an additional disease-induced mortality, the uncertainty introduced by the uncertainty about the most appropriate error model to use, and the estimation of *M* can be addressed. These aspects are detailed further in the Appendix.

Computational aspects

Most Bayesian stock assessments in the published literature have relied either on direct-mapping approaches (e.g., Hilborn and Walters 1992) or on sampling–importance resampling (e.g., McAllister et al. 1994; McAllister and Ianelli 1997). The former approach becomes extremely difficult to implement for computational reasons if there are more than a few parameters, and the latter method requires the specification of an importance function that, for efficiency, should approximate reasonably closely the posterior distribution. Choice of such an importance function is not trivial: McAllister and Ianelli (1997) described eight separate steps in the development of an importance function, each of which requires considerable expertise.

A simpler approach is the Markov chain Monte Carlo (MCMC) method of generating samples from posterior probability distributions, described accessibly by Gilks et al. (1996). Many variants exist, but the method used here and described below was chosen on grounds of simplicity and robustness, albeit at some cost in computational efficiency. A very long Markov chain is calculated of model parameter vector $\boldsymbol{\Theta}$ with a transition kernel defined as conditional posterior probability of each element of $\boldsymbol{\Theta}$, conditional on the other elements. For the transition from iteration $k - 1$ to iteration k in the chain, a randomly chosen element i of $\boldsymbol{\Theta}$ is replaced with a new value drawn from the posterior distribution of $\boldsymbol{\Theta}_i$ con-

Fig. 2. Test of convergence of the Markov chain. Three short chains were started from three different sets of initial parameters, being close to the upper bounds, close to the lower bounds, or else at intermediate values. The chain was assumed to have converged after 1000 iterations. Values of six key parameters are plotted by iteration: (*a*) abundance at age-6 in 1997, (*b*) natural morality, (*c*) abundance at age-14 in 1997, (*d*) spawning stock biomass in 1996, (*e*) proportion of released fish that survive tagging, and (*f*) projected catch for fishing mortality in 1997 = fishing mortality in 1996.

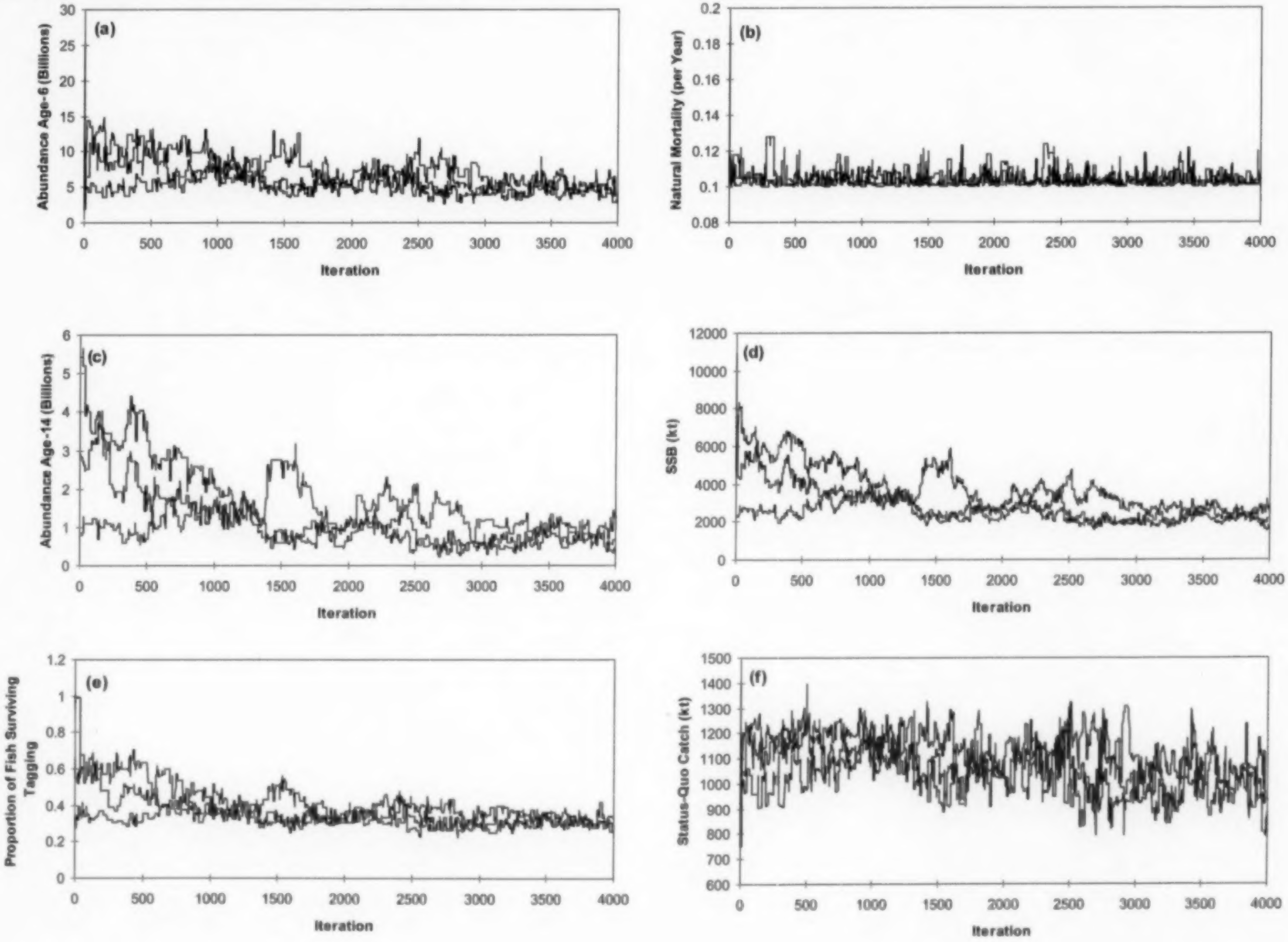
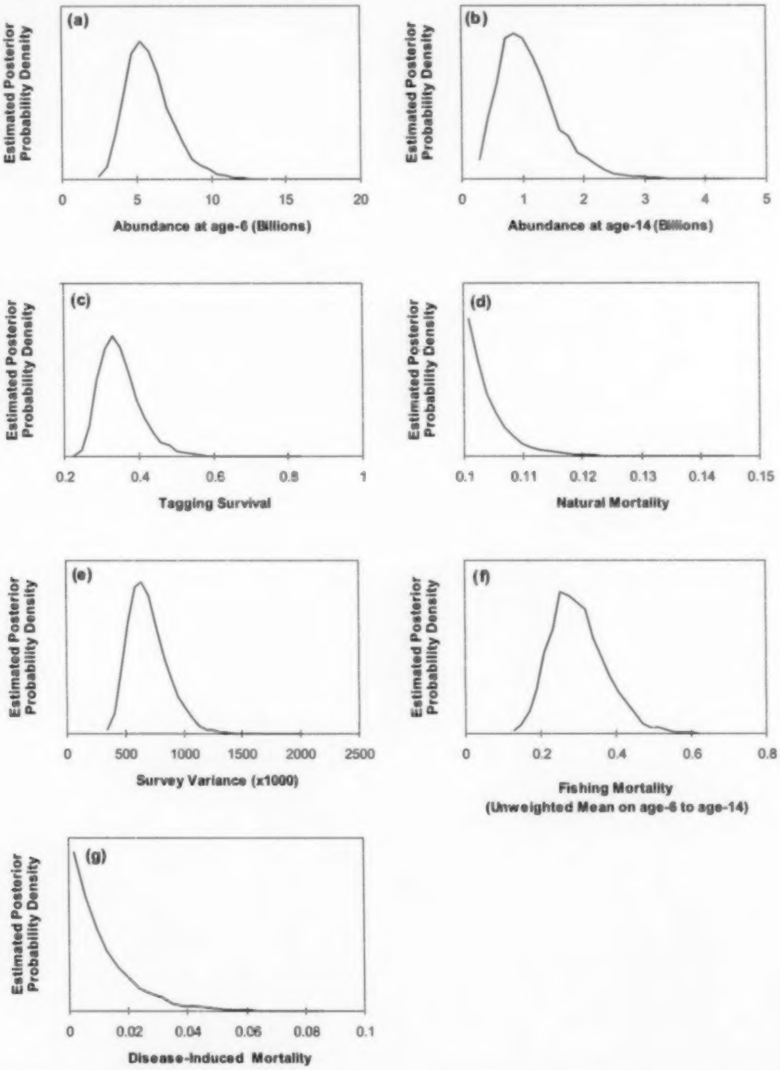


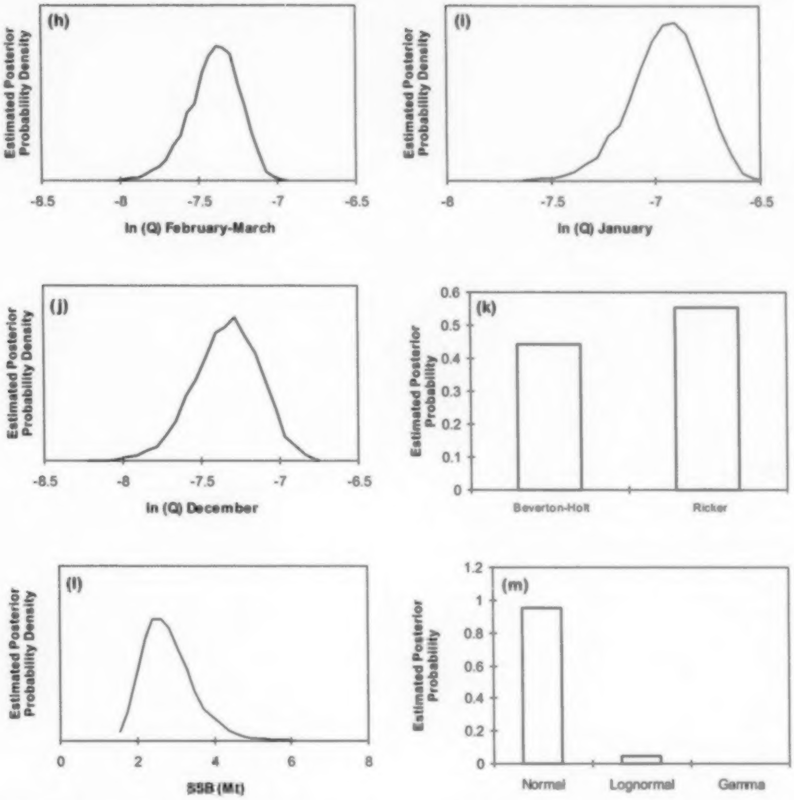
Fig. 3. Estimated Bayes posterior probability distributions for some key parameters in the stock assessment. Note that these figures have no *y*-axis divisions, as the areas under the curves represent unit posterior probability. (*a*) Population abundance at age-6 in 1997; (*b*) population abundance at age-14 in 1997; (*c*) proportion of tagged fish that survive after being released; (*d*) natural mortality of adult fish, excluding disease-induced mortality; (*e*) common variance of all of the acoustic surveys; (*f*) fishing mortality in 1996, as an unweighted mean on age- 6 to age-14; (*g*) additional mortality due to the observed epizootic of *Ichthyophonus hoferi*; (*h*) ln of the coefficient *Q* of proportionality between the VPA population estimates and the estimates from acoustic surveys on prespawning fish in February and March; (*i*) ln of the coefficient *Q* of proportionality between the VPA population estimates and estimates from acoustic surveys on the overwintering populations in January; (*j*) ln of the coefficient *Q* of proportionality between the VPA population estimates and estimates from acoustic surveys on the overwintering populations in December; (*k*) estimated posterior probability of either the Beverton–Holt or the Ricker stock–recruitment model being correct; (*l*) estimated posterior probability density of the spawning stock biomass in 1996; (*m*) estimated posterior probabilities of the normal, lognormal, or gamma error distribution model providing a correct description of the observation error process.



ditional on elements *j* of $\mathbf{\Theta}$ (other than element *i*) remaining unchanged, i.e., drawn from $P(\mathbf{X}|\mathbf{\Theta}_{j,j<>i,k-1}\mathbf{M})P(\mathbf{\Theta}|\mathbf{M})$. Drawing a sample from this conditional likelihood is problematic because the form of the function is not known analytically and it is computationally expensive to evaluate. The approach used here is a modification of the secant method of adaptive rejection sampling described by Gilks (1996).

In this method, sampling for an element of $\mathbf{\Theta} = \mathbf{\Theta}_i$ from the conditional posterior likelihood function $g(\mathbf{\Theta}_{i,k}) = P(\mathbf{X}|\mathbf{\Theta}_{j,j<>i,k-1}\mathbf{M})$ $P(\mathbf{\Theta}|\mathbf{M})$ begins by defining an upper envelope function $(G(\mathbf{\Theta}_i))$. The main criteria for constructing the envelope function are that it should be easy to draw a sample from $G(\mathbf{\Theta}_i)$, it should lie above $g(\mathbf{\Theta}_i)$, and, for computational efficiency, it should be reasonably close to $g(\mathbf{\Theta}_i)$. In this case the upper envelope function is con-

Fig. 3 (*concluded*).



structed as a step function (Fig. 1). Accordingly, sampling from $G(\boldsymbol{\Theta}_i)$ is a simple two-stage process of sampling one from several rectangular distributions, each with a probability mass that is simple to calculate. Once one of the "steps" has been randomly selected, a sample can be drawn from the corresponding uniform distribution.

The initial estimate of $G(\boldsymbol{\Theta})$ is constructed in stepwise fashion between likelihoods estimated at five values of $g(\boldsymbol{\Theta}_i)$ and an estimate of the maximum of the curve (Fig. 1). The central likelihood estimate in the construction is the likelihood in the previous Markov chain step. Two more likelihoods are evaluated at the upper and lower limits of the uniform prior distribution specified for $\boldsymbol{\Theta}_i$ ($\boldsymbol{\Theta}_i^{\mathrm{MAX}}$ and $\boldsymbol{\Theta}_i^{\mathrm{MIN}}$) and a further two likelihoods are evaluated at intermediate values ($\boldsymbol{\Theta}_i^{\mathrm{MIN}} + 0.67(\boldsymbol{\Theta}_{i,k-1} - \boldsymbol{\Theta}_i^{\mathrm{MIN}})$ and $\boldsymbol{\Theta}_i^{\mathrm{MIN}} + 0.33$ $(\boldsymbol{\Theta}_i^{\mathrm{MAX}} - \boldsymbol{\Theta}_{i,k-1})$). In order to make a draw from $g(\boldsymbol{\Theta}_i)$, the first step is to make a draw $\boldsymbol{\Theta}_i^*$ from $G(\boldsymbol{\Theta}_i)$ and to subject it to an accept/reject test: $\boldsymbol{\Theta}_i^*$ is accepted with probability $g(\boldsymbol{\Theta}_i^*/G(\boldsymbol{\Theta}_i^*)$. If the $\boldsymbol{\Theta}_i^*$ is rejected, then the new value of $g(\boldsymbol{\Theta}_i^*)$ is used to update the upper envelope function $G(\boldsymbol{\Theta}_i)$.

The function of the accept/reject test is to correct for the differences between $g(\boldsymbol{\Theta})$ and $G(\boldsymbol{\Theta})$. If $G(\boldsymbol{\Theta})$ is a good approximation to $g(\boldsymbol{\Theta})$ at point $\boldsymbol{\Theta}_i^*$, then $g(\boldsymbol{\Theta}_i^*)/G(\boldsymbol{\Theta}_i^*) \approx 1$ and the draw can be accepted with high probability. If, for example, $G(\boldsymbol{\Theta})$ is a poor approximation to $g(\boldsymbol{\Theta})$ at $\boldsymbol{\Theta}_i^*$ and the upper envelope function is too high, such that $G(\boldsymbol{\Theta}_i^*) = 2g(\boldsymbol{\Theta}_i^*)$, then the draw taken from $G(\boldsymbol{\Theta})$ was taken from a distribution with a probability two times too high at $\boldsymbol{\Theta}_i^*$, and the probability of accepting this value should be 1/2 $(= g(\boldsymbol{\Theta}_i^*)/G(\boldsymbol{\Theta}_i^*))$.

Even if the initial guess of the form of $G(\boldsymbol{\Theta}_i)$ is poor, after a small number of rejections and additions of new function evaluations to the step function, the $G(\boldsymbol{\Theta}_i)$ will usually become sufficiently close to $g(\boldsymbol{\Theta}_i)$ to allow a sample to be accepted. In most cases the process requires about five to eight iterations before a value of $\boldsymbol{\Theta}_i^*$ is accepted. Although computationally not very efficient, this algorithm was robust to structural bound-constraints (such as a restriction of a maximum 100% tagging survival) that this assessment model should logically contain. A Metropolis step was added to ensure robustness of the method to non-log-concave conditional probability distributions (i.e., cases where the conditional probability may emerge above the upper envelope function) (Gilks 1996). In initial trials, mixing and convergence were found to be improved by updating a single, randomly chosen element of $\boldsymbol{\Theta}$ in each iteration rather than attempting to sample the conditional distributions of all elements in each iteration.

The adaptive rejection sampling algorithm serves to sample from the conditional probability distribution of one (continuous) parameter. A much simpler approach serves to take samples from the set of admissible model structures (alternative stock–recruit models or error distributions). Given a set of parameters $\boldsymbol{\Theta}_k$ at a certain point in the chain, then the conditional likelihoods of alternative models (e.g., $\mathbf{M}_{q=1},\ldots, \mathbf{M}_{q=n}$) can be calculated directly as $P(\mathbf{X}|\boldsymbol{\Theta}_k,\mathbf{M}_q),\ldots,$ $P(\mathbf{X}|\boldsymbol{\Theta}_k,\mathbf{M}_q)$. In order to take a sample from the admissible model space, it is then sufficient to draw a sample w (say) from $\mathbf{U}(0,1)$ and to select $\mathbf{M}_{q,k+1}$ for the next Markov chain step which satisfies the condition

$$(3) \qquad \sum\nolimits_{q-1} P(\mathbf{X}|\boldsymbol{\Theta}_k,\mathbf{M}_q) < w < \sum\nolimits_{q} P(\mathbf{X}|\boldsymbol{\Theta}_k,\mathbf{M}_q)$$

since

$$(4)\qquad \sum_{q=1}^{q=n} P(\mathbf{X}|\boldsymbol{\Theta}_k, \mathbf{M}_q) = 1.$$

This approach can be used where all $\boldsymbol{\Theta}$ are common to all $\mathbf{M}$ (e.g., variance parameter common to normal, lognormal, and gamma error models). Where some element of $\boldsymbol{\Theta}$ is model specific (e.g., stock–recruit model parameters), a hierarchical approach is used. Where an element of $\boldsymbol{\Theta}$ is specific to model q, then when evaluating $P(\mathbf{X}|\boldsymbol{\Theta}_k,\mathbf{M}_q)$, that element of $\boldsymbol{\Theta}_k$ is replaced with the most recent value in the chain in which model q was used.

Clearly the adjacent values of $\boldsymbol{\Theta}_k$ in the Markov chain will be very strongly correlated, and this correlation will decrease the further apart in the chain the pairs of values are drawn. For most purposes, we require drawing virtually uncorrelated samples from the chain; a method proposed by Raftery and Lewis (1996) allows calculation of the appropriate intervals from which to take samples from the chain so that they have less than a specified correlation (for the Norwegian spring-spawning herring example, this was calculated as one sample each 76 iterations for 5% accuracy in the posterior distributions). This process is sometimes known as "thinning" the chain.

The chain will also clearly be sensitive to starting conditions, and one will wish to discard a certain number of initial iterations, after which the chain is effectively independent of the starting choices for $\mathbf{M}$ and $\boldsymbol{\Theta}$. This is sometimes known as the "burn-in" period. From the Raftery and Lewis (1996) estimation method, this was estimated as 600 iterations. A further check on the convergence properties of the chain was made by restarting several short chains from different starting parameters and plotting the development of some key parameters through the first few thousand iterations, as suggested by Gelman (1996). This calculation was made to seek to identify problems of the type reported by Gelman and Rubin (1992). Sequences so obtained are given in Fig. 2. A visual inspection of these chains suggests that a 600-iteration burn-in period may be an underestimate; hence, when the method was applied, the first 1000 iterations were discarded.

In order to improve robustness of the parameter distributions to possible parameter correlations and also to reduce the influence of starting values, the parameter estimates were drawn from the chain at intervals of 100 iterations, and 7500 samples were taken from a chain of over 750 000 iterations. In order to avoid the recalculation of the burn-in and thinning parameters for the chain, after a few initial trials, a value of 1000 for the burn-in and a value of 100 for thinning were used. Posterior distributions are approximated by plotting the frequencies of the parameters in around 7500 samples (implying a chain length of 751 000 iterations).

Values of $\boldsymbol{\Theta}'$ can be saved from a single run of the chain in order to reevaluate the outcome of various harvest control laws without incurring the computational burden of reevaluating the entire chain.

Assessment model and harvest control law

A stock assessment model was formulated as described that is similar to that described in Anonymous (1997*c*). This model is of conventional ADAPT type, with inclusion of tag return data and an assumption of Poisson errors.

It would conceptually be preferable to use a population model that does not rely on the assumption of error-free catch-at-age observations rather than a simple VPA approach (e.g., Fournier and Archibald 1982), and this would be more appropriate to the treatment of the other observations. However, where the assumption of precise catches-at-age is relaxed, it must be replaced with some structural assumption about selection pattern. It is not clear what selection model would be appropriate for this stock because much of the catches taken has relied on a few abundant cohorts on which the fisheries have actively been targeting. Therefore, for this stock the simple VPA population model has been retained. For stocks with a stable selection pattern, the MCMC method described here can in principle be used in conjunction with a statistical catch-at-age model (e.g., Anonymous 1998). Three observation error modds (normal, lognormal, and gamma) were allowed with equal probability, and both Beverton–Holt and Ricker stock–recruit relationships were allowed with equal probability. Natural mortality was assumed unknown with equal probability between specified bounds (0.1–0.2). In addition, the possibility of a disease-induced fish kill was admitted by allowing additional mortality on the affected year-classes in the range 0–0.1 with equal probability in the period when the outbreak was observed. The MCMC implementation described above was used to generate draws of the key stock assessment parameters $\boldsymbol{\Theta}$ (listed in the Appendix).

A harvest control law was suggested by Anonymous (1997*d*) in which catches are regulated as a function of a precautionary fishing mortality F_{pa} (which takes the value of the conventional $F_{0.1}$ parameter), the estimated current spawning stock size (SSB), a precautionary biomass B_{pa} below which F is reduced, and a limit biomass B_{lim} that defines a low and unacceptable level of biomass that managers seek to avoid by implementing the harvest control law.

In the proposal by Anonymous (1997*b*), the B_{pa} and B_{lim} reference points are defined in terms of the estimated biomass B_{MSY} at which a deterministic maximum sustainable yield (MSY) can be removed from the stock. The three reference points defined for the harvest control law are

$$\mathbf{T} = (B_{\text{pa}} = B_{\text{MSY}},\ B_{\text{lim}} = 0.5B_{\text{pa}},\ F_{\text{pa}} = F_{0.1})$$

and the harvest control law $\mathbf{H}(\mathbf{M},\boldsymbol{\Theta},\mathbf{X},\mathbf{T})$ determining harvest in year y is defined as follows.

$$\text{If } \mathrm{SSB}_{y-1} > B_{\text{pa}}, \text{ then harvest}_y = \text{catch for } F = F_{\text{pa}}.$$

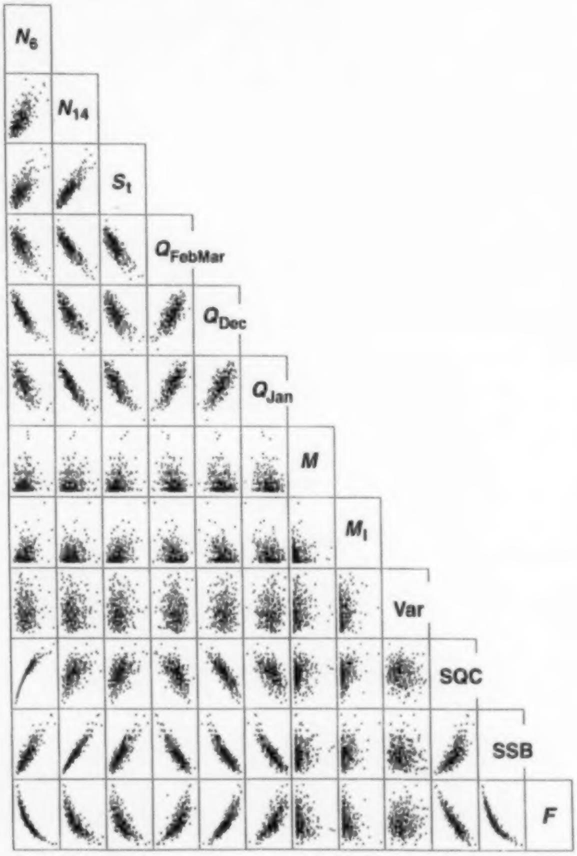
$$\text{Otherwise, if } \mathrm{SSB}_{y-1} > B_{\text{lim}}, \text{ then harvest}_y = \text{catch for } F = F_{\text{pa}}(\mathrm{SSB}_{y-1} - B_{\text{lim}})/(B_{\text{pa}} - B_{\text{lim}}).$$

$$\text{Otherwise, if } \mathrm{SSB}_{y-1} < B_{\text{lim}}, \text{ then harvest}_y = 0.$$

Estimates of the reference points for the harvest control law are dependent on $\boldsymbol{\Theta}$, $\mathbf{M}$, and $\mathbf{X}$, and hence, uncertainty in the reference points depends on the $P(\boldsymbol{\Theta},\mathbf{M}|\mathbf{X})$. Here, $F_{0.1}$ is estimated conventionally from $\mathbf{M}$ and the weights-at-age and the exploitation pattern. In order to investigate the sensitivity of the precision of the $F_{0.1}$ estimate to uncertainty in recent selection, distributions have been calculated using either (*i*) the selection pattern in 1996, which is obviously highly uncertain, or (*ii*) the mean selection pattern over the whole period 1960–1996, which is much more stable.

For present purposes, B_{MSY} has been estimated as half the maximum observed stock size in the time series (this depends on making an assumption of the Schaefer form of stock production and relies on low F in the early part of the time series). This approach was used instead of using estimates of B_{MSY} derived from an age-structured production modelling approach because for this stock, the estimates of historic stock and recruitment tend to lie around a straight line passing through the origin. Thus, the data do not robustly define a maximum recruitment within the range of historic observations, and attempts to derive MSY estimates based on such stock–recruitment models were considered to be inappropriate. Clearly, B_{MSY} depends very much on $\mathbf{M}$ and on $\mathbf{X}$. Estimates of the distributions of future recruitments are made dependent on drawn recruitment models ($\mathbf{M}_r$, say, a component of $\mathbf{M}$) and model-specific pairs of drawn parameters $\boldsymbol{\Theta}_r|\mathbf{M}_r$. A two-stage procedure is used in which uncertainty in $\mathbf{M}_r$ and $\boldsymbol{\Theta}_r$ is modelled, as above, by

Fig. 4. Multivariate scatterplot of 400 samples from the Markov chain of parameters. N_6 and N_{14}, cohort abundances at age- 6 and age-14 on 1 January 1997; S_t, tagging mortality; Q_{FebMar}, Q_{Dec}, and Q_{Jan}, acoustic survey catchabilities for the surveys on the spawning grounds in February and March, for surveys on the wintering grounds in December, and for surveys on the wintering grounds in January; M, natural mortality; M_I, additional natural mortality caused by a disease outbreak; Var, acoustic survey variance (pooled for all surveys); SQC, F status quo catch forecast for 1997; SSB, spawning stock size in 1996; F, fishing mortality in 1996.



MCMC draws from the posterior distribution. Uncertainty due to stochastic noise, the variability in recruitment by year around a given recruitment model defined by $\mathbf{M}_r$ and $\mathbf{\Theta}_r$, is modelled using a nonparametric bootstrap method. For each draw of $\mathbf{M}_r$ and $\mathbf{\Theta}_r$, a set of fitted historic recruitments R_y can be calculated ($\mathbf{R}_y = f(\mathbf{M}_r, \mathbf{\Theta}_r, \mathrm{SSB}_y)$). From the same draw, a set of historic recruitments $N_{0,y}$ can be derived, dependent on the drawn M and other parameters through the usual VPA assumptions. A set of recruitment residuals specific to the drawn $\mathbf{M}_r$ and $\mathbf{\Theta}_r$ can then be calculated as

$$(5)\qquad \varepsilon^r_y = \ln(R_y/N_{0,y}).$$

Future recruitments in year y are modelled as

$$(6)\qquad N_{0,y} = g(\mathbf{M}_r, \mathbf{\Theta}_r, \mathrm{SSB}_y)\exp(\varepsilon^r_*)$$

where ε^r_* indicates a random draw with replacement from ε^r_y.

As defined above and including the procedure for modelling forecast recruitment, **T** depends on **X**, **Θ**, and **M** and a harvest control law catch depends directly on **X**, **Θ**, **M**, and **T**. Hence, for any drawn **Θ** and **M**, a catch corresponding to the harvest control law can be calculated. Making this calculation repeatedly for all the drawn **Θ** and **M** from the MCMC process allows the posterior distribution for the catch conforming to the harvest control law **H** to be constructed.

Results

Estimated posterior probability distributions for stock assessment parameters **Θ**, model choices **M**, and some dependent variables that are of particular management interest are given in Fig. 3. These indicate rather wide confidence intervals for the cohort abundances and catchabilities, with SSB estimates for 1996 in the region 2–4 Mt. The distribution of M was rather collapsed onto the lower bound of the uniform prior range, suggesting $M = 0.1$ or below. The tagging survival model suggests between 30 and 40% survival of tagged fish. The data did not appear to allow much discrimination between Beverton–Holt and Ricker stock–recruit models, for which the estimated posterior probabilities (0.45 and 0.55) where close to the prior probabilities (0.5 and 0.5). However, a normal error distribution appeared more likely than either the lognormal or gamma distributions in this case. There was little indication of significant disease-induced mortality.

Some indication of the correlation structure of the assessment model can be obtained from a multivariate scatterplot of some of these variates (Fig. 4). This shows marked positive correlations between cohort abundances, tagging survival, SSB, and status quo catch estimates and marked negative correlations between SSB and F (as one would expect) and also between the three catchability parameters and estimates of cohort abundance and stock size. Other parameters (M, survey variance, disease-induced mortality) did not appear highly correlated with any other parameters, although this could be due to the rather restricted range of M investigated here. This rather suggests that improvements in the precision of estimates of stock size, F, or status quo catch could be made by imposing informative prior distributions on the survey catchabilities rather than on any other parameters.

Estimated probability densities for the $F_{0.1}$ and B_{lim} reference points are given in Fig. 5. The distribution of the B_{lim} reflects its dependence on M and the constraints imposed on that parameter. The distribution of 1997 stock size (subject to the harvest control law) spreads from below B_{lim} to above B_{pa}, with a mode at about $1.3B_{\mathrm{lim}}$. Two distributions of $F_{0.1}$ were calculated, based either on the 1996 weights-at-age and exploitation pattern or on means from 1960 to 1996. The former distribution is both wider than the latter and located at higher values of F. It is wider because there is more uncertainty in the 1996 exploitation pattern than in the historic mean exploitation pattern, and it is located at a higher F because the 1996 exploitation pattern does not include any exploitation of young fish, which did occur in the early part of the time series. The distribution of F in 1996 relative to the $F_{0.1}$ estimate (based on the 1996 weights-at-age and exploitation pattern) is centred around three to four times $F_{0.1}$.

The probability distributions for the harvest control law catches (Fig. 6) in 1997 and 1998 indicate that rather low

Fig. 5. Estimated probability densities (*a*) of B_{lim}, (*b*) of $F_{\text{pa}} = F_{0.1}$ reference points and the associated probability densities of stock size in 1997, (*c*) of fishing mortality in 1996, and (*d*) relative to those reference points. Two calculations of $F_{0.1}$ are made, based either on 1996 stock weights and selection pattern or on mean weights and selection in the period 1960–1996.

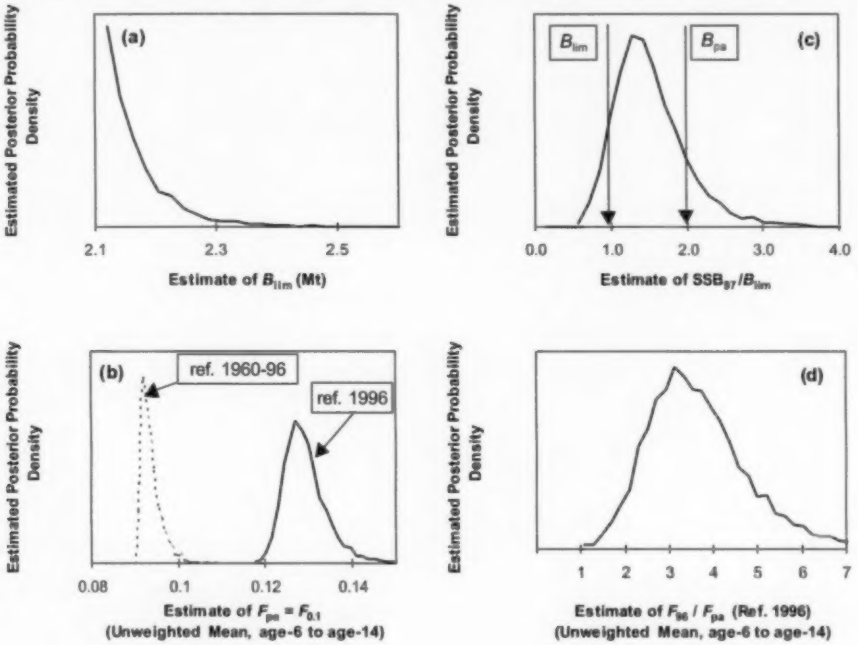


Fig. 6. Estimated probability densities of harvest control law catches for (*a*) 1997 and (*b*) 1998 and cumulative probability that a given catch will exceed the catch corresponding to the harvest control law in (*c*) 1997 and (*d*) 1998. Probability distributions are calculated using the estimates of $F_{0.1}$ using the estimate of the exploitation pattern in 1996.

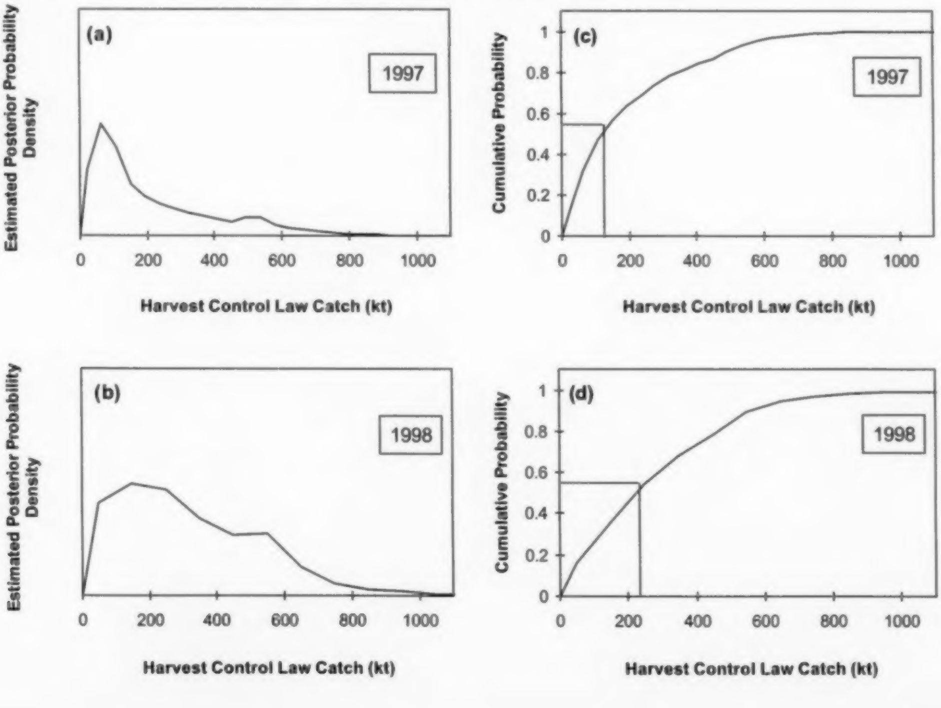
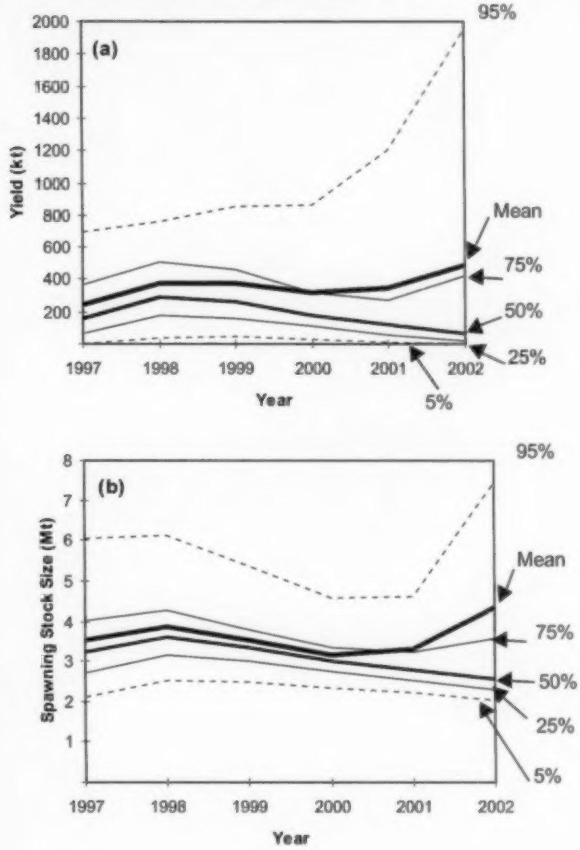


Fig. 7. Perceptions of future catches and future stock size in the medium term resulting from the implementation of the harvest control law (*a*) yield and (*b*) spawning stock size.



catches of the order of 120 000 t have a 50% chance of conforming to the harvest control law in 1997, but this value increases to some 220 000 t in 1998. However, given the asymmetric nature of the probability distribution of the harvest control law catch, it may be more appropriate to base advice on the means of the distributions, = 215 000 t for 1997 and 324 000 t for 1998 in this example.

An example of this sort of calculation is given in Fig. 7, which illustrates the perception of stock development from the stock modelling and harvest control law procedure described above. This example shows an interesting feature in that the distributions of catches and stock size become increasingly asymmetric with time: the median and the arithmetic mean of the distributions diverge increasingly with time.

For comparison, the MCMC uncertainty estimates have been compared with estimates derived from a conventional parametric bootstrap method. Assuming firstly a normal error structure for the surveys, a maximum-likelihood model fit was calculated. Based on this fit, 3000 pseudo data sets were constructed with mean values equal to the expected values for the surveys and variance as calculated in the maximum-likelihood fit. Tag experiment pseudo data were constructed similarly, with draws made from Poisson distributions. The assessment model was fitted to each pseudo data set, with bound constraints as imposed by the limits of the uniform prior distributions. Variances were calculated conditionally on the other parameters. The procedure was repeated with the assumption of lognormal and gamma survey error distributions in addition to the normal assumption. A summary of parameter distributions so obtained is given in Fig. 8. Note that nearly all the iterations finished on the bound constraint of $M = 0.1$.

Discussion

Admitting uncertainty in error structure clearly increases the perception of uncertainty in stock size. Parametric bootstrap estimates under the three error model assumptions are widely different (expected stock sizes of 2.03, 1.64, and 2.4 Mt (normal, lognormal, and gamma)) and each has a much lower uncertainty estimate attached than the Bayesian distribution. Perhaps surprisingly, the Bayes distribution is wider than the overlap of the three parametric bootstrap distributions would indicate. This may be due to the conditional nature of the bootstrapping procedure.

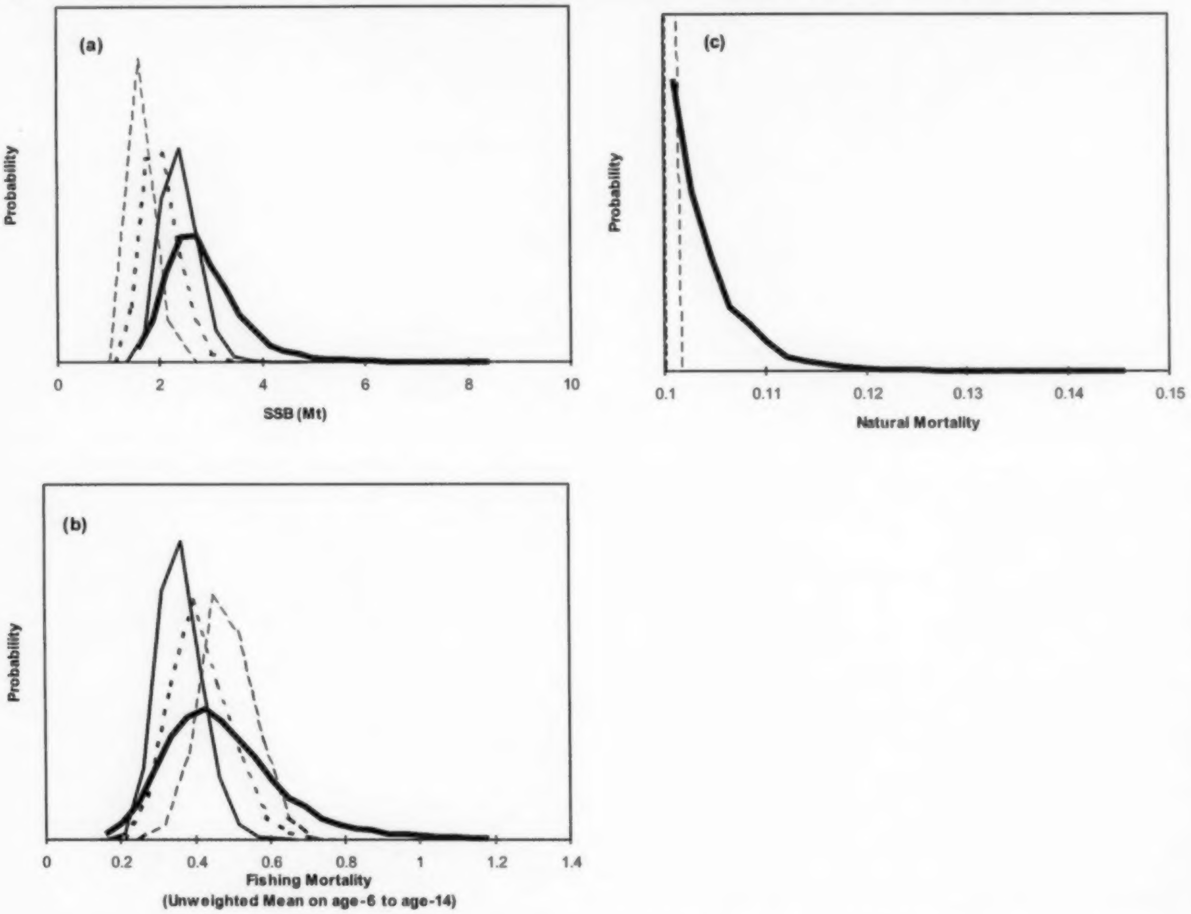
The estimated probability distribution for M (Fig. 3*d*) is surprising in that it appears closely bound constrained at a value of 0.1/year. This may indicate that M in this stock is considerably lower than has been believed, but may also indicate that despite the inclusion of the tag return observations, the estimate of M derived using the structural model used here may not be stable. At present, it is not known which of these alternatives is more credible, but further analyses of the stability of estimates of M to alternative model structures are considered necessary.

The approach used here to evaluate harvest control law catches is quite different from conventional methods. Usually one would consider the $\mathbf{H}$ as a quota-setting rule that is applied by a manager in response to current perceptions of $\mathbf{M}$ and $\boldsymbol{\Theta}$. Here, however, $\mathbf{H}$ is considered as a process to which the manager is attempting to approximate. The manager may do so by setting a quota according to a perception of the outcome of the application of $\mathbf{H}$ while taking account of uncertainties in the various inputs to $\mathbf{H}$.

This approach has the advantage of putting together the presently somewhat diverse processes of stock assessment, estimation of reference points, medium-term projections, and management simulation into a single modelling procedure, which has observations, model structures, definitions of biological reference points, and prior perceptions defined clearly as separate inputs. This procedure allows calculation of a harvest control law catch in probabilistic terms in a consistent fashion and can include uncertainty in M and other uncertainties as appropriate. Simple extensions can allow uncertainty in model structure to be modelled also (type of stock–recruit relationship, nature of error distributions, etc.). Conversely, it allows advice to be given on the catch to be taken in the short term to conform to a given harvest control law with specified probability or to calculate an expected catch for a given harvest control law.

The method could also be used to compare outcomes of proposed harvest control laws. Using a conventional management simulation procedure allows evaluation of a harvest

Fig. 8. Comparison of probability distributions for some key parameters obtained using either the Markov chain Monte Carlo approach or a parametric bootstrap method for estimating uncertainty. (*a*) Distribution of spawning stock biomass in 1996; (*b*) distribution of fishing mortality in 1996; (*c*) distribution of natural mortality. The parametric method was repeated assuming that acoustic survey errors have a normal, lognormal, or gamma distribution. Thick solid line, Bayes posterior probability distribution estimate; short-dashed line, conditional parametric bootstrap, normal errors assumed for surveys; long-dashed line, conditional parametric bootstrap, lognormal errors assumed for surveys; thin solid line, conditional parametric bootstrap, gamma errors assumed for surveys.



control law **H** given beliefs about the stock dynamics **M** and **Θ**. A harvest control law evaluation is typically a lengthy process of evaluating different combinations of **H** and **M** while ignoring **X** and **Θ**. Arguably, a more attractive approach would be to specify beliefs about **M** and **Θ** as prior distributions as described above. This would have the advantage that the beliefs would be updated using the observation data set **X**. Reevaluation of medium-term prospects for stock development for alternative **H** when new information is obtained that may alter perceptions of **M** and **Θ** can then be a rather simple and routine task.

Recently, it has become a requirement from managers that assessment scientists should provide estimates of the uncertainty with which management-related quantities such as future stock size or catch are estimated, under a specified harvesting rule such as a specified F rate (Anonymous 1996*c*, 1997*a*, 1997*b*). This is so that managers may take decisions based on the perceived risks of following a particular course of action. Arguably, however, present practice in fisheries assessment science is such that the estimates of uncertainty that are routinely calculated are largely incomplete. There are two reasons for this. The first is that structural uncertainty, the probability that a different model structure should apply, is neglected. The second is that uncertainty in M is usually neglected. A relatively simple calculating mechanism is proposed here that allows such uncertainties to be evaluated formally, even when applied in cases where it is necessary to evaluate the outcome of complex harvest regimes.

Risk calculations are usually made on the basis of quantifying stochastic noise in the data around a stock assessment model that is assumed to be correct. However, a brief consideration of cases where large revisions in perceptions of stock size have occurred (e.g., Pacific anchovy (Csirke 1988), northern cod (Walters and Maguire 1996), North Sea herring (Anonymous 1995), western mackerel (Anonymous 1979)) suggests that large uncertainties (and therefore large risks) are not due to stochastic noise but rather to mistaken perceptions of appropriate model structure. These arose due to insufficient understanding of biological or technological processes.

An objective and realistic consideration of uncertainty would therefore encompass an evaluation of the risks that any of a large number of plausible alternative assessment models could be correct. In practice, the suite of possible alternative models is likely to be so large that a truly comprehensive and objective calculation can be made, and some subjective restriction on the range of allowable models must be made. As a claim to objectivity cannot reasonably be made, it is preferable to use a Bayesian class of statistical analysis in which admissible structural models are defined a priori, and these are then treated in a consistent fashion throughout the procedure of stock assessment and uncertainty evaluation.

This approach has the advantage of making a clear distinction between observation and assumption. It allows probability distributions for quantities of management interest to be calculated relatively easily even where these quantities may depend on intermediate parameters, such as biological reference points, in a complex way. Because of the availability of cheap and fast computers, Bayesian computational methods for integrating under problems of large dimensionality can now provide a convenient means for quantifiying uncertainty while incorporating a wider range of structural uncertainties than has hitherto been customary. Here, such a procedure is described and is applied to a case of the assessment of Norwegian spring-spawning herring. It is suggested that this approach provides improved uncertainty estimates for management purposes and should therefore be particularly useful where an implicit management goal is to seek to maintain a specified level of risk.

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Appendix. Observations, prior distributions, and model structures for the assessment of Norwegian spring-spawning Atlantic herring

Observations

The observations used here are the conventional stock assessment data taken from Anonymous (1997*c*). They comprise the estimates of annual landings L_y and (separately by age group a and by year y) numbers of fish caught ($C_{a,y}$), their mean weight in catches ($\mathrm{CW}_{a,y}$) and in the stock ($\mathrm{SW}_{a,y}$), and the proportion of fish spawning $O_{a,y}$. These are estimated from sampling of commercial catches. Data from 1950 to 1996 and of age-0 to age-16+ were used.

Three series (j) of acoustic survey measurements ($U_{a,y,j}$) of the stock size exist, taken by surveying overwintering aggregations in December and January or on prespawning aggregations in February and March.

Tagging experiments intended to measure fish mortality F have been carried out on an annual basis since 1975 (Anonymous 1996*c*). The *i*th "experiment" here is defined as the release of $K_{i,y}$ fish in year y and all the subsequent recaptures from that release. In years after the release, a number of fish (m_y) were screened for tags in the commercial catches $C_{a,y}$. The number of tagged fish of each age recaptured was recorded ($X_{i,a,y}$).

The data are taken from Anonymous (1997*c*).

Structural models

Axiomatic structural model elements

The set of possible structural models is extremely large. For present purposes, a set of relationships are defined as axiomatic, in that they are assumed to hold throughout and all conclusions drawn are predicated on the assumption that these hold. Other structural relationships in which uncertainty is admitted are termed "candidate structural models." The model structures here treated as axiomatic are those that have been used as assumed structures by Anonymous (1997*c*) and are widely accepted in the stock assessment field.

Underlying population structural model

The population structural model is Pope's (1972) cohort analysis model. This is chosen in preference to the usual Baranov equation virtual population analysis model merely on the grounds of faster computation. Selection pattern constraints were imposed as described in Anonymous (1997*c*).

Acoustic survey catchability model

The expected acoustic survey stock size estimates are assumed to be related to population abundance by a "catchability" coefficient Q specific to each survey time series j as

$$\text{(A1)} \quad E(U_{j,a,y}) = Q_j N_{a,y}.$$

Tag returns model

As recaptures of tagged fish are extremely rare events, a Poisson error distribution has been assumed, following Hilborn and Walters (1992). Assume that of $K_{i,y}$ fish tagged, a proportion S_t will survive the process of tagging, release, and recapture. This proportion is common to all tagging experiments. Further, assume that after the tagged fish have suffered the initial mortality, the fish then suffer no additional mortality and can then be considered to have the same dynamics as the untagged fish. Obviously, it is not possible to know the age of a fish at the time of tagging, since it must be released alive, but in the case of this stock the 1983 cohort has been exceptionally strong, and it is possible to make rather unambiguous assignations to age merely from length–frequency information. For present purposes, only tagging information from this cohort is considered.

Defining the abundance of tagged fish at large as $T_{a,y}$, we have for the first year after release

$$\text{(A2)} \quad T_{i,a,y} = K_{i,y} \mathrm{S}_t$$

and in subsequent years

$$\text{(A3)} \quad T_{i,a+1,y+1} = T_{i,a,y} \exp\left(-M - \mathrm{MI}_{a,y} - F_{a,y}\right).$$

As only a minuscule fraction of the stock is tagged, the impact of the tagging experiment on the untagged population N is not modelled. From the usual catch equation, the expected number of tag returns from the *i*th experiment will be

$$\text{(A4)} \quad \hat{W}_{i,a,y} = \left(\frac{m_y}{\sum_a C_{a,y}}\right) T_{i,a,y} \frac{F_{a,y}}{F_{a,y} + M + \mathrm{MI}_{a,y}} \times \left(1 - \exp\left(-F_{a,y} - M - \mathrm{MI}_{a,y}\right)\right).$$

Under assumption of Poisson errors, the logarithm of the likelihood of observing the tag returns $W_{i,a,y}$ given the structural models as above is

$$\text{(A5)} \quad \sum_{i,a,y} W_{i,a,y} \ln(\hat{W}_{i,a,y}) - \hat{W}_{i,a,y} - \ln(W_{i,a,y}!).$$

Tag model returns appeared to conform well to both the structural models and an assumption of Poisson errors. A single additional parameter S_t is estimated and is assumed equal for all tagging experiments.

Candidate model elements

For present purposes, uncertainty in the observation error structure for the acoustic surveys is admitted, and also uncertainty in the appropriate form of stock–recruit relationship.

Acoustic survey error models

The three candidates tested are that the acoustic surveys estimates are distributed with normal, lognormal, or gamma errors, with expectation given as above. The three forms of log-likelihood function corresponding to each are as follows.
Normal:

$$\text{(A6)} \quad -\frac{1}{2}\sum\left(\ln(2\pi) + \ln(\sigma^2) + \frac{(U_{a,y,i} - Q_i N_{a,y})^2}{\sigma^2}\right)$$

Lognormal:

$$\text{(A7)} \quad -\frac{1}{2}\sum_{a,y,i}\left(\ln(2\pi) + \ln(U_{a,y,i}^2 \sigma_i^2) + \frac{\ln(U_{a,y,i}/Q_i N_{a,y})^2}{\sigma_i^2}\right)$$

Gamma:

$$\text{(A8)}\quad -U_{j,a,y}/\beta_{j,a,y} + (\alpha_{j,a,y} - 1)\ln(U_{j,a,y}/\beta_{j,a,y}) - \ln(\beta_{j,a,y}\Gamma(\alpha_{j,a,y}))$$

where

$$\beta_{j,a,y} = \sigma^2/(q_j N_{a,y} \exp((-F_{a,y} - M_{a,y})t_j))$$

$$\alpha_{j,a,y} = (q_j N_{a,y} \exp((-F_{a,y} - M_{a,y})t_j)^2)/\sigma^2$$

$$\Gamma(\alpha_{j,a,y}) = \int_0^\infty \exp(-u)u^{(\alpha_{j,a,y}-1)}\mathrm{d}u.$$

The gamma model was included in addition to the normal and lognormal models used previously by Anonymous (1996*a*) on account of a perception that there are marked residual patterns in opposite directions when the two foregoing models are fitted. Such trends are apparent when standardized residual plots around maximum-likelihood model fits are compared. The gamma model is intermediate between those two models in terms of the change in variance with fitted value.

Stock–recruitment models

Five different forms of recruitment models were considered by Anonymous (1996*a*), and their implications for medium-term stock development were considered. Here, only two of these forms have been admitted as acceptable alternatives.

(*i*) Beverton–Holt form with lognormal error. The structural model is

$$\text{(A9)}\quad R_y = \frac{A \cdot \mathrm{SSB}_y}{B + \mathrm{SSB}_y}$$

and the corresponding log-likelihood function is

$$\text{(A10)}\quad -\frac{1}{2}\sum\nolimits_{a,y,i}\left(\ln(2\pi) + \ln(R_y^2\sigma_r^2) + \frac{\ln(R_y/N_{0,y})^2}{\sigma_r^2}\right)$$

(*ii*) Ricker form, assuming lognormal error. The structural model is

$$\text{(A11)}\quad R_y = A \cdot \mathrm{SSB}_y \exp(-B \cdot \mathrm{SSB}_y)$$

and the observation error model remains as above.

Scalar parameters and priors

Referring to the Ricker form above, in the present model the parameter vector $\boldsymbol{\Theta}$ contains the unknown scalars (1) population abundance in 1997, age-6 ($N_{6,1997}$), (2) population abundance in 1997, age-14 ($N_{14,1997}$), (3–5) the catchabilities Q for each of the three acoustic surveys, (6) the initial tagging mortality S_t, (7) natural mortality M, (8) the additional mortality MI due to *Ichthyophonus*, (9) the variance of the acoustic surveys σ^2, and (10–11) parameters of the stock–recruit relationship, and the alternative model structures **M** tested are (1) normal, lognormal, or gamma probability density function for acoustic surveys and (2) the Beverton–Holt or Ricker form of the stock–recruit relationship.

Natural mortality

In the case of Norwegian spring-spawning herring, it is hypothesized that there may have been an additional mortality from an outbreak of the disease *Ichthyophonus hoferi*, which has been reported between the years 1991 and 1994. Consequently, additional mortality due to the disease outbreak (MI) and mortality from other natural causes (M) are treated as separate model parameters. The disease outbreak has been observed only to affect fish of certain ages in certain years. Based on these observations, the natural mortality model (as used by Anonymous 1996*a*) can be written

Natural mortality = M, unless $y > 1990$ and $y < 1995$ and $(y - a) > 1987$, when $M = M + \mathrm{MI}$.

In Anonymous (1996*a*), M and MI take assumed values of 0.13 and 0.10, respectively.

Nuisance parameters

The survey catchabilities Q_j, the survey variances σ^2, and the stock–recruit relationship variances σ_r^2 are not necessarily of interest for management purposes. Walters and Ludwig (1994) proposed analytic solutions for eliminating these from the calculation of Bayesian posterior distributions, but their findings are specific to Gaussian distributions. Here, numerical integrations over the variances and catchabilities Q are used, and using the Markov chain Monte Carlo method the numerical demands of the calculations become tractable.

Prior distributions

Uniform prior distributions were chosen for each alternative model structure, on the basis that no model is known a priori to be more likely than any other (e.g., P(lognormal errors) = 0.33; P(Ricker stock–recruit = 0.50)). Uniform prior distributions were also chosen for population abundance parameters 1 and 2 and 9–11 above, with range chosen to be sufficiently wide to define regions of virtually zero posterior probability.

Restrictive prior distributions were chosen for M, MI, and tagging survival. The prior distribution used for the tagging survival parameter S_t was uniform from 0.2 to 1.0. This parameter must have an upper bound set to 1 for obvious reasons.

A uniform prior distribution for MI was chosen based on testing the estimate used by Anonymous (1996*a*) of MI = 0.1, so that a uniform distribution over the range 0.0–0.1 was used. Similarly, the range for the baseline M was chosen as 0.1–0.2, centred on the Working Group assumption of $M = 0.15$.

A reciprocal prior distribution for the survey variance was used (prior probabilities proportional to $1/\sigma^2$), according to Jeffreys (1961), with a range chosen to be unrestrictive.

Larval fishes of a Middle Atlantic Bight estuary: assemblage structure and temporal stability

David A. Witting, Kenneth W. Able, and Michael P. Fahay

Abstract: We collected weekly, quantitative ichthyoplankton samples over 6 years (1989–1994, 1309 samples) to identify temporal scales of variability in the abundance and occurrence of larval fish assemblages near Little Egg Inlet in southern New Jersey, U.S.A. We collected species that spawn in the estuary (30%), both the estuary and continental shelf (35%), continental shelf (25%), and the Sargasso Sea (10%). The following analyses suggest an annually repeated seasonal progression of species assemblages: (*i*) the rank abundance of the 20 dominant species did not change significantly from year to year, (*ii*) variation in the density of the dominant species was primarily explained by intraannual rather than interannual variation, and (*iii*) multivariate analysis of the assemblage matrix identified five seasonal assemblages that occurred during all six years. We found that the timing and duration of each of these seasonal groups were correlated with two characteristics of the annual temperature cycle, magnitude (higher or lower temperature) and trajectory (increasing vs decreasing temperature). We suggest that the repeated occurrence of larval fish assemblages in temperate estuaries along the U.S. coast may, in part, be driven by local environmental processes.

Résumé : Nous avons prélevé hebdomadairement des échantillons quantitatifs d'ichtyoplancton durant 6 ans (1989–1994, 1 309 échantillons) pour établir les échelles temporelles de variabilité de l'abondance et de la présence de groupements de larves de poissons près du bras Little Egg dans le sud du New Jersey, aux États-Unis. Nous avons recueilli des espèces qui frayent dans l'estuaire (30%), à la fois dans l'estuaire et sur la plate-forme continentale (35%), sur la plate-forme continentale seulement (25%) et dans la mer des Sargasses (10%). Les résultats suivants laissent penser qu'il y a une progression saisonnière répétée annuellement des groupements d'espèces : (*i*) les abondances relatives des 20 espèces dominantes n'ont pas changé significativement d'année en année, (*ii*) la variation de la densité de l'espèce dominante s'expliquait principalement par une variation intra-annuelle plutôt qu'inter-annuelle et (*iii*) l'analyse multivariée de la matrice des groupements a permis de repérer cinq groupements saisonniers qui étaient présents durant les six années. Nous avons observé que le moment de formation et la durée de chacun de ces groupements saisonniers étaient corrélés avec deux caractéristiques du cycle annuel de la température, soit le degré de température (température basse ou élevée) et la tendance de la température (température croissante ou décroissante). Nous pensons que la survenue répétée des groupements de larves de poissons dans les estuaires tempérés de la côte américaine peut en partie être déterminée par des processus environnementaux locaux.

[Traduit par la Rédaction]

Introduction

The orderly seasonal succession in abundance and species composition of the dominant components of temperate estuarine assemblages figures prominently in all general reviews (Allen and Barker 1990; McGovern and Wenner 1990). Geographical variation in sources of larvae is also a common characteristic of many estuarine fish assemblages (Frank and Leggett 1983; Suthers and Frank 1991; Hettler and Barker 1992). There are, however, few published examples that demonstrate the repeatability of species assemblages and abundances from year to year. A large part of these seasonal changes have been attributed to the transient nature of the dominant species such that the estuary is only used during specific life history stages (McGovern and Wenner 1990). Others have suggested that spawning is timed such that the arrival of early life history stages into the nursery grounds takes advantage of favorable conditions relative to feeding, predation, etc. (e.g., Frank and Leggett 1983), thus producing a seasonal pattern in the species composition of larvae entering the estuary. In either case, long-term data that test the pattern of repeated progression of seasonal assemblages are largely unavailable.

In this paper, we consider larval supply and the temporal stability of estuarine fish assemblages by examining an ichthyoplankton assemblage in the polyhaline portion of a southern New Jersey, U.S.A., estuary with weekly sampling over 6 years. In this estuary, as in others, each year of observations involves a new year-class of all species involved; thus, the influence of interannual variation in larval supply can be clearly identified. If the larval fishes occur in repeated, predictable sequence, we may infer that larval supply may stabilize an annual pattern of species abundances. Alternatively, if the seasonal sequence of species occurrence varies from year to year, or if larval absolute and rank abundances

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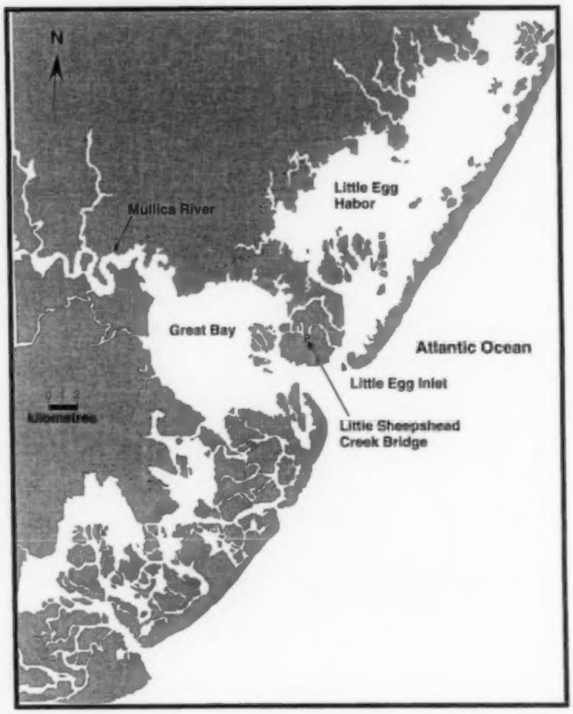
Fig. 1. Great Bay – Little Egg Harbor estuary in southern New Jersey with the sampling location at Little Sheepshead Creek near Little Egg Inlet.



vary dramatically from year to year, we can infer that larval supply is a source of interannual variation in the seasonal progression of species.

Methods

Study area

The Great Bay – Little Egg Harbor estuarine system (Fig. 1) is polyhaline and shallow (average depth 1.7 m). It comprises a drowned river valley (Mullica River), embayment (Great Bay), and adjacent barrier beach estuary (Little Egg Harbor). A natural inlet (Little Egg Inlet) is the primary source of ocean water entering this estuary. Several thoroughfares or "creeks", including Little Sheepshead Creek, run through a peninsula and serve to connect Great Bay and Little Egg Harbor. This estuary shares many characteristics with other estuaries in the Middle Atlantic Bight including a broad seasonal temperature range (–2 to 28°C) and a moderate tidal range (about 1 m) (Able et al. 1992). The fauna of resident and migratory fishes is enriched by northern and southern migrant species (Able and Fahay 1998).

All samples of larval and early juvenile fishes, hereafter referred to as larvae, were collected from a bridge that spans Little Sheepshead Creek (Fig. 1). The bridge is located 3 km from the creek mouth, 2.5 km from Little Egg Inlet. Water depth at the sampling location is about 4 m. Atlantic Ocean water flows into the estuary through Little Egg Inlet during flood tides, some directly into the mouth of Little Sheepshead Creek.

Sampling protocol

We collected samples weekly by suspending a 1-m-diameter (1-mm mesh) plankton net from the bridge during night flood tides (referred to hereafter as a deployment). Five 30-min deployments of two nets, one at the surface and one near the bottom, produced a total of 10 deployments per sampling date beginning in February 1989. Between May 1990 and July 1991, we made three deployments of two nets at these same depths, and between August 1991 and November 1994, we made three deployments of one net halfway between surface and bottom (midwater). There was no shift in species abundance (relative, absolute, or rank) in the year that we changed from paired surface and bottom deployments to a single midwater deployment. To estimate the volume of water sampled, we fixed a General Oceanics flowmeter in the mouth of the net. We made a total of 42 deployments without flowmeters between 1 and 17 February 1989 and 1309 deployments with flowmeters between 20 February 1989 and 30 November 1994. We did not include samples from deployments that were made without flowmeters in any analyses of density. We sampled an average of 401 m^3 (150 m^3 SD) of water in each tow, and a small fraction (5%) of deployments sampled less than 100 m^3. At the beginning and end of each sampling effort, we measured surface water temperature with a field thermometer and salinity with a refractometer. To determine long-term interannual variation in temperature, we also incorporated a data set of daily water temperatures recorded in the estuary spanning 18 years (Able et al. 1992).

After each deployment, we sorted all samples in the laboratory by placing small portions of the samples in shallow pans and removing all fish, which were then preserved in 95% ethanol. We then identified, measured, counted, and assigned a notochord flexion stage to each preserved fish. We used notochord flexion to characterize the onset of the transition from larval (preflexion) to juvenile (postflexion) life history stages. Lengths were also used to indicate ontogenetic state; however, we consider flexion stage to be a better indicator than length due to the potentially confounding effects of shrinkage that may occur due to preservation. We considered larvae to be in the preflexion stage if the notochord was straight at the caudal tip, flexion stage if the notochord flexed dorsally at the caudal peduncle and hypural formation had begun, and postflexion stage if the hypural plate was fully formed.

There were a few taxonomically confusing forms. For example, *Ammodytes* may be represented by two species, but was most likely *Ammodytes americanus* because of the estuarine sampling location (Nizinski et al. 1990). In addition, we did not resolve the identification of two species of goby (*Gobiosoma bosc* and *Gobiosoma ginsburgi*) until the third year of sampling (Duval and Able 1998); thus, we combined them as *Gobiosoma* spp. for the 1989 and 1990 samples.

Data analysis

We used nested ANOVA (Hicks 1993) to partition the temporal variation in density of each of the 20 most dominant species into interannual, intermonth, and interdate variation, testing for the contribution of each temporal scale of variability (year, month within year, and date within month and year) to the total variation in the density. The density estimates for each species were log transformed prior to analysis, and density estimates from each deployment were used as the sampling unit. We included only the months that a species was collected (all years combined) and examined interyear versus intrayear variation within and between these months; we first established the months during which each species was collected by averaging the densities and generating 95% confidence intervals and then included densities within these confidence limits. After conducting the nested ANOVA, we also calculated the fraction of the variation explained by each temporal scale of variability from the total variation explained by the model (R^2) and the average density and the coefficient of variation (CV) for each species collected.

We conducted analyses that tested for temporal consistency in

Table 1. Sample size and flexion stage for the 20 most abundant forms collected from Little Sheepshead Creek in the vicinity of Little Egg Inlet.

Species	Total no.	Cumulative %	Spawning area	No. staged	Preflexion (%)	Flexion (%)	Postflexion (%)
Anchoa mitchilli	105 117	77.6	E, C	4 399	6.3	27.3	66.2
Syngnathus fuscus	7 056	82.8	E	2 165	0	0.1	99.2
Menidia menidia	4 486	86.1	E	1 434	11.3	24.2	64.2
Pleuronectes americanus	3 750	88.9	E	1 801	6.9	84.1	8.7
Ammodytes sp.	2 352	90.6	C	1 018	6.0	34.2	59.8
Anguilla rostrata	2 238	92.2	SS	1 359	0	0	100
Clupea harengus	1 427	95.1	C	1 047	0	0.7	99.3
Gobiosoma bosc	1 082	95.9	E	607	0	3.6	96.4
Fundulus heteroclitus	942	96.6	E	246	0.4	0	99.6
Brevoortia tyrannus	870	97.2	E, C	623	0.2	2.4	97.4
Etropus microstomus	724	97.8	C	445	0	0	100
Paralichthys dentatus	671	98.3	C	433	0	0	100
Scophthalmus aquosus	434	98.6	E, C	306	27.1	39.5	31.1
Gasterosteus aculeatus	346	98.9	E	199	0	0	100
Tautoga onitis	289	99.1	E, C	131	0	21.4	78.6
Conger oceanicus	284	99.3	SS	163	0	0	100
Micropogonias undulatus	244	99.5	C	185	0	16.2	83.8
Gobiosoma ginsburgi	235	99.7	E, C	235	0.4	0.9	98.7
Gobionellus boleosoma	222	99.8	E, C	163	0	0	100
Cynoscion regalis	203	100	E	127	6.3	15	68.5
Total	132 972			17 086	3.2	14.8	82.5

Note: Spawning areas are estuaries (E), continental shelf (C), or Sargasso Sea (SS) based upon Able and Fahay (1998).

Table 2. Results of nested ANOVA testing for the contribution of years, months within years, and dates within months and years to the total variation in density of the 20 species most frequently collected from Little Sheepshead Creek in the vicinity of Little Egg Inlet.

Species	R^2	Date within month and year (%)	Month within year (%)	Among years (%)	Month range	Average density	Interyear (CV)
Anchoa mitchilli	0.75***	32.5***	62.4***	5.2 ns	6–11	216.730	78.10
Syngnathus fuscus	0.76***	28.3***	71.7***	<0.1 ns	3–7	15.927	87.70
Menidia menidia	0.76***	47.1***	52.9***	<0.1 ns	4–12	9.079	86.10
Pleuronectes americanus	0.69***	54.5***	45.5**	<0.1 ns	4–5	8.445	61.6
Ammodytes sp.	0.72***	48.7***	51.3***	<0.1 ns	3–5	4.203	72.20
Anguilla rostrata	0.59***	25.6***	74.4***	<0.1 ns	11–6	3.17	69.70
Clupea harengus	0.71***	23.8***	13.3***	62.9***	1–4	2.53	153.30
Gobiosoma bosc	0.63***	68.4***	31.6*	<0.1 ns	6–11	3.009	51.50
Fundulus heteroclitus	0.54***	77.6***	22.0**	0.5 ns	1–12	1.156	137.40
Brevoortia tyrannus	0.54***	65.4***	34.6***	<0.1 ns	8–6	1.374	39.10
Etropus microstomus	0.60***	64.4***	34.7**	0.9 ns	7–11	1.698	83.90
Paralichthys dentatus	0.57***	46.1***	48.7***	5.3 ns	10–6	1.031	70.00
Scophthalmus aquosus	0.77***	79.6***	13.7 ns	6.8 ns	5–7	0.943	97.40
Gasterosteus aculeatus	0.54***	79.3***	20.7**	<0.1 ns	8–6	0.462	63.50
Tautoga onitis	0.79***	79.6***	7.6 ns	12.8 ns	6–7	0.730	214.20
Conger oceanicus	0.56***	88.9***	<0.1 ns	11.1 ns	5–6	0.502	84.50
Micropogonias undulatus	0.44***	70.8***	29.2*	<0.1 ns	9–12	0.414	77.60
Gobiosoma ginsburgi	0.50**	36.2 ns	<0.1 ns	63.8*	7–8	0.698	129.60
Gobionellus boleosoma	0.71***	57.9***	<0.1 ns	42.1***	9–11	0.365	213.70
Cynoscion regalis	0.61***	92.8***	7.2 ns	<0.1 ns	6–8	0.472	86.70

Note: R^2 refers to the proportion of the variation in the density of each species explained by each level. Date within month and year refers to the percentage of the variation explained by variation among dates, month within year refers to the percentage of the variation explained by variation among months, and among years refers to the percentage of the variation explained by differences between years. ns, not significant ($p > 0.05$); $^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

Fig. 2. (*a*) Composite length–frequency distribution of all fish (n = 22 795) measured from ichthyoplankton collections from Little Sheepshead Creek in the vicinity of Little Egg Inlet. Lengths are all standard length except for Anguilliformes, which are total length. (*b*) Frequency distribution of interyear CV for all species collected.

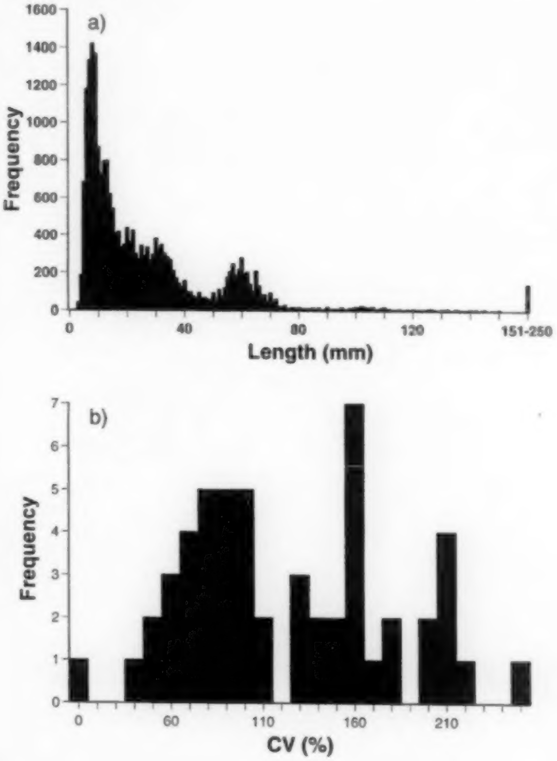
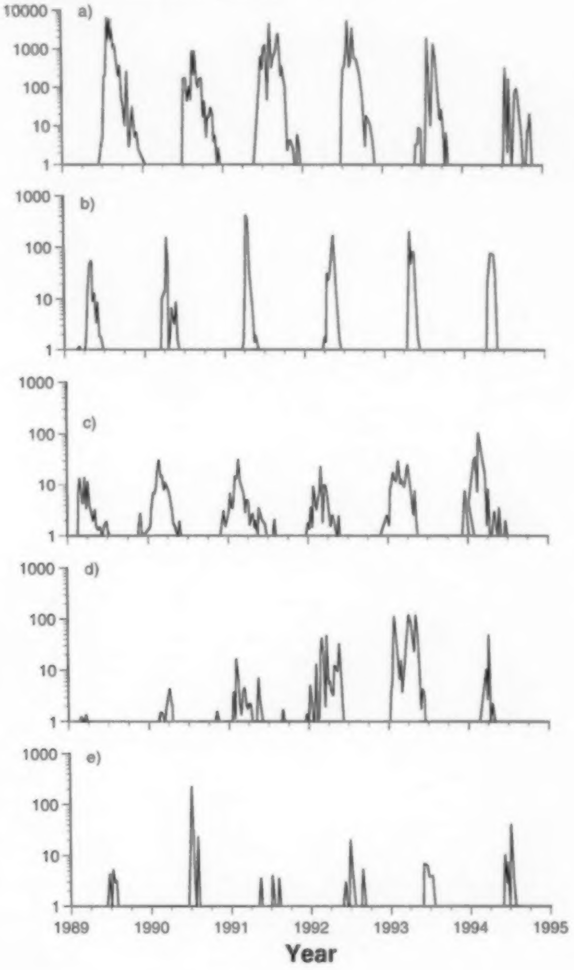


Fig. 3. Density plotted against date for five fish species collected from Little Sheepshead Creek in the vicinity of Little Egg Inlet. (*a*) Summer estuarine spawner (*Anchoa mitchilli*); (*b*) spring estuarine spawner (*Pleuronectes americanus*); (*c*) Sargasso Sea spawner, winter immigrant (*Anguilla rostrata*); (*d*) shelf spawner, winter immigrant (*Clupea harengus*); (*e*) summer estuarine spawner (*Fundulus heteroclitus*).



assemblage structure using rank correlation analysis (Sokal and Rohlf 1981). We ranked the 20 dominant species in order of their abundance for each year from highest to lowest and then generated a correlation matrix that tested the null hypothesis that the ranking of a species in one year was not correlated with its ranking in another year.

To identify seasonal assemblages of species, we used cluster analysis. We used a faunal distance metric, cord normalized expected species shared (CNESS) (Trueblood et al. 1994), which is related to both Orloci's (1978) chord distance and Grassle and Smith's (1976) faunal similarity index (normalized expected species shared (NESS)), as a transformation that balanced the contribution of rare and dominant species. This index circumvents the assumption of a specific distribution of individuals among species in nature and the dependence upon sample size (Sanders et al. 1980). The probabilities of sampling species k from sample i given a random draw of m individuals from sample i were calculated by

$$H_{iklm} = \frac{1-[(N_i - N_{ik})Cm]}{(N_i Cm)}$$

where $[N_i, C, m]$ is the combination function, or the number of unique ways of sampling m objects from a sample of N_i individuals; N_i was calculated by

$$[N_i, C, m] = \frac{N_i!}{[m!(N_i - m)!]}$$

The Euclidian distance between samples in the centered, normalized H matrix (Pielou 1984) is the CNESS index. Finally, we used group-averaged cluster analysis (Pielou 1984) to group the samples into seasonal assemblages (Trueblood et al. 1994). To analyze these data, we calculated CNESS using an m size of 10.

To determine the relationship between the timing of each seasonal assemblage (as defined by cluster analysis) and temperature, we used smoothed daily surface temperatures from the estuary. We calculated an average and a daily rate of change from the daily temperatures recorded for the 10 days prior to each day of sampling. With these two measures of temperature (magnitude and trajectory), we identified the occurrence of each assemblage type over

Table 3. Rank abundance for each year and average rank for the 20 most common species collected in Little Sheepshead Creek in the vicinity of Little Egg Inlet and year-by-year correlation matrix for rank species abundance.

Species	1989	1990	1991	1992	1993	1994	Mean ± SE
Anchoa mitchilli	1	1	1	1	1	2	1.17±0.17
Syngnathus fuscus	2	2	4	2	2	1	2.17±0.40
Pleuronectes americanus	4	4	2	4	5	6	4.17±0.54
Gobiosoma spp.	5	5	5	3	4	7	4.83±0.54
Menidia menidia	3	8	3	7	9	3	5.50±1.15
Ammodytes sp.	6	3	6	11	12	4	7.00±1.51
Anguilla rostrata	7	7	7	9	7	5	7.00±0.52
Clupea harengus	8	16	10	5	3	9	8.50±1.84
Etropus microstomus	9	14	9	8	6	12	9.67±1.17
Brevoortia tyrannus	10	9	11	10	13	13	11.00±0.68
Paralichthys dentatus	12	11	8	13	16	10	11.67±1.12
Fundulus heteroclitus	11	6	18	15	15	14	13.17±1.70
Scophthalmus aquosus	13	18	17	16	8	8	13.33±1.82
Conger oceanicus	16	15	16	18	10	11	14.33±1.28
Anchoa hepsetus	15	10	13	19	11	20	14.67±1.69
Tautoga onitis	14	17	20	6	20	16	15.50±2.13
Cynoscion regalis	17	20	14	12	18	15	16.00±1.18
Gasterosteus aculeatus	18	12	15	17	17	17	16.00±0.89
Micropogonias undulatus	19	13	19	14	14	19	16.33±1.20
Gobionellus boleosoma	20	19	12	20	19	18	18.00±1.24
1994	0.89	0.9	0.64	0.79	0.69	0.73	
1993	0.78	0.78	0.55	0.67	0.65		
1992	0.83	0.83	0.53	0.66			
1991	0.84	0.84	0.69				
1990	0.78	0.78					

the seasonal pattern in temperature. For example, spring may be similar to fall in magnitude, but opposite in trajectory. We used a two-factor ANOVA to test for the relationship between year, assemblage type, mean preceding temperature, and trajectory of temperature change.

Results

Environmental patterns

The seasonal pattern of water temperatures in spring, summer, and fall was consistent among years. Temperature ranged from –2 to 27°C (annual average 12.7°C) during the 6 years of sampling. The timing of temperatures rising above the annual average in the spring and falling below the annual average in the fall was always within 15 days of 1 May and 1 November. Summer temperatures typically peaked at 20–25°C, with very little interyear variability in the pattern. Winter temperatures dropped to about 0°C and showed higher levels of interannual variability.

Salinity ranged from 22 to 32‰ over 6 years, with an average salinity of 28.6‰ (±0.16 SE). Salinity did not show a consistent seasonal pattern, but did vary significantly within sampling periods. Salinities prior to the first deployment of the net on each date were significantly lower than those after the last deployment (27.7 versus 29.3‰) (ANOVA, F = 31.4, p = 0.0001), indicating that higher-salinity ocean water was entering the sampling site with the flooding tide.

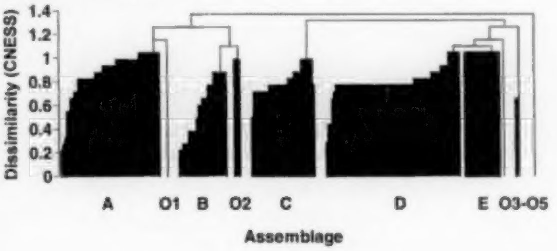
Species and life history stage composition

A total of 135 528 fish representing 81 species in 43 families were collected during the sampling period. The dominant families were Bothidae (six species), Gadidae (five), and Sciaenidae (five). The most abundant species was *Anchoa mitchilli* (77.6% of all fish collected). Five species made up 90.6% of all larvae, and the top 20 species made up 98.1% (Table 1). Estuarine species that were absent from these collections were those with demersal larvae (e.g., *Opsanus tau*) and those that occur primarily in the oligohaline portions of the estuary (e.g., *Trinectes maculatus*).

Fish of all life history stages (larval to adult) from 2 to 250 mm were collected during the study (Fig. 2*a*). The majority, however, were larvae and early juveniles between 4 and 75 mm. The aggregate length–frequency distribution revealed two distinct modes. The first mode, at about 10 mm, comprised primarily *Pleuronectes americanus* (28%) and *A. mitchilli* (21%). A second mode, at about 60 mm, comprised *Anguilla rostrata* "glass eels" and *Conger oceanicus* late leptocephali (68%).

All flexion stages were observed in the dominant species (Table 1). Overall, the collections were composed primarily of postflexion individuals (82.5%), with most of the others undergoing flexion (14.8%) and few in the preflexion stages. There was considerable variation between species in the proportion of each stage that was collected. Of the 20 most frequently captured forms, *Gobionellus boleosoma*, *G. bosc*, *G. ginsburgi*, *Clupea harengus*, *Brevoortia tyrannus*, *Fundulus heteroclitus*, *Gasterosteus aculeatus*, *Paralichthys denta-*

Fig. 4. Results of cluster analysis of CNESS similarities that identify five distinct seasonal assemblages (A–E) and five smaller outliers (O1–O5).



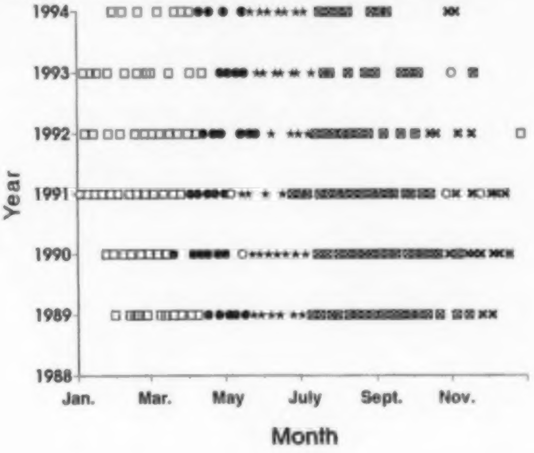
tus, *Etropus microstomus*, and *Syngnathus fuscus* were almost entirely postflexion larvae or juveniles. In six of the abundant species (*A. mitchilli*, *Menidia menidia*, *P. americanus*, *Ammodytes* sp., *Tautoga onitis*, and *Micropogonias undulatus*), a large fraction of the individuals were undergoing flexion. In contrast with most of the abundant species collected, *Scophthalmus aquosus* were collected at all flexion stages.

Temporal stability and assemblage structure

Most of the 20 dominant species showed higher levels of variability within years than between years (nested ANOVA) (Table 2). Dominant species typically occurred as a pulse, reaching similar maximum densities in each year (Fig. 3). This pattern was true for species that spawn locally as well as for those that spawn far from the estuary (Figs. 3*a*–3*c*). In all but *G. ginsburgi*, the component of the variation in density explained by sampling dates within years was highly significant (Table 2). CV's for interyear abundance (calculated for all species represented by more than 10 individuals) varied considerably among species and ranged from 39 to 245% (Fig. 2*b*). The distribution of CV's was lognormal (Wilk–Shapiro, $W = 0.96$, $p > 0.10$). Of the 20 most frequently captured forms, all but five (*G. ginsburgi*, *C. harengus*, *G. boleosoma*, *T. onitis*, and *F. heteroclitus*) had CV's that were lower than the 50th percentile of the lognormal distribution. Other forms showing lower interyear CV's were *Prionotus carolinus*, *Prionotus evolans*, *O. tau*, and *Myrophis punctatus*. Of the five species that showed higher CV's (see above), four also had higher variance components at the interyear temporal scale. For example, interyear variation explained a higher (12.8%) but nonsignificant portion of the variation in *T. onitis*. *Clupea harengus* increased in abundance each year during the sampling period (Fig. 3*d*) and *G. boleosoma* was absent in one year, leading to greater interyear variation than intrayear variation. The fifth species with a high CV (*F. heteroclitus*) was collected in extreme abundance on a single day in 1990 (Fig. 3*e*).

Larval assemblage structure was consistent from year to year (Table 3). The rankings for the 20 most common species for all years were significantly correlated ($p < 0.05$). Correlation coefficients for year-by-year comparisons ranged from 0.53 to 0.90 and were normally distributed (Wilk-Shapiro, $p > 0.5$). In addition, there is no evidence of change over the 6-year time series (each year was equally similar to adjacent years as to more distant years). Most species were collected in each year. Seventeen of the 20 dominant species were collected in all six years. Thirty species (31.6%) were collected in either five or six years. Twenty- eight species (29.5%) were collected in only one year. These were all rare species, and 24 of them were represented by a single specimen.

Fig. 5. Seasonal progression of species assemblages for each of the six years sampled. Each cluster type is represented by a different symbol. Assemblages, based upon results of cluster analysis, include major (A (□), B (●), C (★), D (⊠), E (×)) and outlier (○) assemblages. Note the consistent seasonal progression among years and the timing of outlier assemblages during the transitional periods between major assemblages.

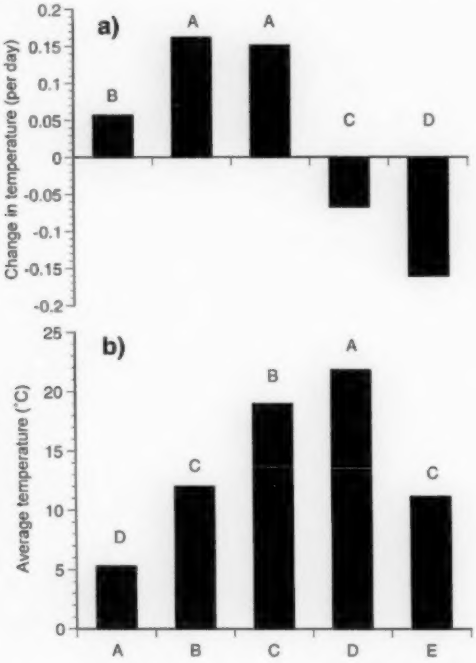


CNESS similarity and group average analyses resulted in five distinct seasonal groups and five samples that were different (referred to hereafter as outliers) from the main clusters (Fig. 4). Four of the five seasonal assemblage occurred in each year (Fig. 5). Outliers (designated O1–O5) arose between seasons and represented seasonal transitions (Fig. 4). The only exception to the general seasonal progression occurred in 1993, when the fall assemblage E did not occur.

A unique assemblage of species dominated each major cluster/assemblage. *Clupea harengus* and *A. rostrata* dominated the late winter assemblage A, with lower numbers of *Ammodytes* sp., *P. dentatus*, and *B. tyrannus*. *Pleuronectes americanus* and *Ammodytes* sp. dominated the early spring assemblage B, in addition to lower numbers of *C. harengus*, *A. rostrata*, and *B. tyrannus*. *Menidia menidia*, *S. fuscus*, and *A. mitchilli* dominated the late spring assemblage C, with smaller numbers of *S. aquosus*. *Anchoa mitchilli* dominated the summer assemblage D that made up 96% of the total number of larvae in the summer samples. Finally, the fall assemblage E was the most diverse group, with strong representation by *B. tyrannus*, *M. undulatus*, *P. dentatus*, and *A. mitchilli* and smaller numbers of *M. punctatus*, *Urophycis regia*, *Symphurus* sp., *Fundulus majalis*, *E. microstomus*, and *A. rostrata*. Rare species composed a larger fraction of the fall group (6.7%) than any of the other seasonal assemblages (0.6–2.2%).

Temperature influenced the timing and duration of the assemblages. The average temperature varied significantly among the six years sampled, largely due to interannual variation in winter temperatures. Mean temperatures and

Fig. 6. Results of an SNK multiple range test that tested for differences among major assemblages (A–E on the horizontal axis) in (*a*) average temperature and (*b*) average temperature trajectory. Bars labeled with similar letters are not significantly different.



temperature trajectories varied significantly among the six assemblage types identified by the cluster analysis (Fig. 6). The year by assemblage interaction was not significant. Multiple comparisons (SNK) tests demonstrated that each seasonal assemblage occurred during a unique combination of temperatures and temperature trajectories (Fig. 6). Where mean preceding temperatures were similar between assemblage types (B versus E), the rate of temperature change was similar in magnitude, but opposite in direction (Fig. 6). When the rate of temperature change was similar in direction and magnitude (B versus C), the average preceding temperatures were significantly different (Fig. 6).

Discussion

Life history stage and species composition

The species composition of larval fishes that we collected in this estuary was characteristic of the juveniles and adults that are collected in the polyhaline portion of the system (Able and Fahay 1998). The size and stage information for the dominant species indicates two general categories of fishes. The first is characterized by abundant smaller, earlier-stage larvae that were often caught over a larger size range as well. These are primarily local spawners (e.g., *P. americanus*, *A. mitchilli*, and *S. aquosus*). The second is characterized by typically larger, later-stage fishes that either spawn farther outside the estuary and are transported there (e.g., *A. rostrata*, *C. oceanicus*, *C. harengus*, *B. tyrannus*, and *P. dentatus*) or hatch at an advanced stage of development (e.g., *F. heteroclitus*, *S. fuscus*, and *Hippocampus erectus*). The dominance of postflexion stages for other species that spawn in the estuary (e.g., *G. bosc* and *G. boleosoma*) is explained by transport of newly hatched larvae soon after hatching to low-salinity portions of the estuary (Massmann et al. 1963; Shenker et al. 1983) and by net mesh selectivity.

The species that entered the estuary from distant spawning locations were doing so during the transformation to the juvenile stage; thus the shift from oceanic to estuarine habitats was coupled with a morphological change from larva to juvenile. These species represented a diversity of taxa. *Clupea harengus* and *B. tyrannus* entered the estuary largely as late-larval, postflexion individuals. Similarly, two of the abundant flatfish, *E. microstomus* and *P. dentatus*, entered the estuary at a narrow size range as postflexion larvae and primarily after the onset of eye migration. *Conger oceanicus* entered the estuary as late-stage leptocephali and *A. rostrata* as glass eels. Estuarine entry during the transition from the larval to juvenile stage is characteristic of many estuarine assemblages of the South Atlantic Bight (Hoss and Thayer 1993), the Gulf of Mexico (Yanez-Arancibia et al. 1985), Spain (Arias and Drake 1990), South Africa (Beckley 1986), and Australia (West and King 1996). We collected a larger range of stages of more locally spawned species because of their greater availability at all stages of development. The best examples are *S. aquosus* and *M. menidia*, but this was also true for *P. americanus*, *Ammodytes* sp., and *A. mitchilli*.

Each analysis indicates a distinct seasonal progression in species composition. The annual pattern in species assemblages is highly correlated with temperature. Each segment of the seasonal temperature curve likely contributes unique habitat attributes (dissolved oxygen levels, primary productivity, species and size distribution of predators, etc.), thus creating temporal partitioning of resources (sensu DeAngelis and Waterhouse 1987). Similarly, strong seasonal patterns in species composition have also been identified in systems where the seasonal temperature variation is greater than the interannual variation. Warlen and Burke (1990) found a consistent winter and spring assemblage of larval fishes in a North Carolina estuary that were similar to the fall, winter, and spring collections reported here, with *B. tyrannus*, *P. dentatus*, *M. undulatus*, and *A. rostrata* being the dominant species. Several studies conducted in North Carolina (e.g., Warlen 1994) and in New Jersey found that larval *B. tyrannus* appeared in the estuaries during the fall and spring, as they did in our samples. *Brevoortia tyrannus* is an important component of two seasonal assemblages, fall and late winter. This may be a result of two groups of larvae entering the estuary, those that are spawned locally arriving in the fall and those that are spawned more distantly arriving in the spring (S.M. Warlen, K.W. Able, and E. Laban, unpublished data). The Great South Bay (New York) spring and summer fish larval species composition was similar to those presented here for the Great Bay – Little Egg Harbor with dominance of anchovies, gobies, syngnathids, atherinids, and labrids in the summer and *A. americanus*, *P. americanus*, and *S. aquosus* among the dominant species in the fall (Monteleone 1992). In our study, and others conducted in estuaries in the western North Atlantic Ocean (e.g., Pearcy and Richards 1962; Vouglitois et al. 1987), *A. mitchilli* is the dominant species during the summer.

Temporal stability in assemblage structure

Our analyses of the larval fish assemblages of the Great Bay – Little Egg Harbor estuary suggest a high degree of temporal predictability at seasonal and annual scales. A predictable seasonal progression of species assemblages reoccurred in each of the six years studied. Almost all of the dominant species not only occurred during all years sampled, but their rank order of abundance was also consistent among years. Furthermore, our analysis of variation in the densities of individual species also suggests that interannual patterns in the Great Bay – Little Egg Harbor estuary appeared to be predictable. In general, interannual variability did not contribute significantly to the total variation in density of most of the abundant fish species (Table 2).

Three species (*C. harengus*, *G. boleosoma*, and *G. ginsbergi*) varied significantly in density among years. *Gobiosoma ginsburgi* was combined with *G. bosc* for the first 2 years of sampling so that there were fewer years of data for this species; this smaller sample size may explain the increased interannual variation. *Clupea harengus* and *G. boleosoma* represent northern and southern species that are at the extremes of their ranges. In addition, interannual variation in *C. harengus* may be a result of the dramatic increase in western Atlantic stocks of this species (Smith and Morse 1990), which results in a consistent increase in abundance during the six years that we sampled (Fig. 3*d*). There was also a notable difference between the relatively high interyear CV (137.4%) and the relatively small interyear variance component for *F. heteroclitus*. It is likely that the high CV for *F. heteroclitus* is probably due to the large numbers caught on 6 July 1990 (>500, 10 times as many as on any other date (Fig. 3*e*)), resulting in an increase in variation among dates within years, rather than among years.

Only a few studies have attempted to quantify interyear variability in abundance of larval fishes. This is because most larval surveys span about 1 year (but see Allen and Barker 1990). Longer-term studies are becoming more widely recognized as important in understanding estuarine processes (Wolfe et al. 1987) where the importance of understanding the temporal scale within which variation occurs is emphasized (Livingston 1987). Monteleone et al. (1987) attempted to determine the interyear variability in *A. americanus* over a 32-year period by combining a large number of sampling programs. Their sampling was inconsistent throughout the period, creating gaps in the data; thus, they were unable to partition inter- and intra-year variability. In the only study similar to the one presented here, Allen and Barker (1990) also determined that interannual variability over a 5-year period did not contribute significantly to the total variability of most of the larval fishes in the North Inlet estuary, South Carolina.

Among the dominant species collected in the Great Bay – Little Egg Harbor estuary, within-year variation (among months or sampling dates) accounted for the most variation in density. Variability in density within seasons could be explained by variation in the timing of pulses of larvae entering the estuary from distant spawning areas (Frank and Leggett 1983; Allen and Barker 1990). Estuarine spawners should therefore show less variability within a season than those from distant spawning areas. In this estuary, variation among months was the highest for the estuarine-spawning species (mean 63.2%), lowest for continental shelf spawners (mean 49.1%), and intermediate for continental shelf/estuarine spawners (mean 53.9%) (Table 2). The relationship between intrayear variability and spawning location may be complicated by estuarine- or inlet-specific processes that effect the specific timing of spawning, hatching, or transport. The variation in the timing of entry into the estuary from distant spawning grounds may be, in part, determined by local, episodic oceanographic events such as upwelling or downwelling that are known to occur at this site (Neuman 1996).

Allen and Barker (1990) found that the higher proportion of the variation in density of winter species compared with summer was explained by interyear variation. In the present study the few species that showed significant interyear variability were both winter- (*C. harengus*) and summer-spawned species (*G. ginsburgi* and *G. boleosoma*). All fish that showed significant interyear variability were near the extremes of their range and possibly their physiological limits (sensu Miller et al. 1991).

In summary, the seasonal pattern of estuarine larval fish assemblages in temperate east coast estuaries similar to our study site may be driven by factors both internal and external to the estuary. For many species the timing, and perhaps location, of spawning is set such that larvae that are transported to estuarine nursery grounds are likely to encounter specific environmental conditions that vary among species. The fishes that utilize the Great Bay – Little Egg Harbor estuary as nurseries are spawned in a variety of locations ranging from local estuarine spawning to very distant spawning in the Sargasso Sea. The geographic variation in spawning locations also produces variation in the dependence upon local processes for dispersal during the larval period among species (Suthers and Frank 1991). There is, however, evidence here that local processes do play a major role dictating the timing of arrival of estuarine fishes, even those that are spawned in areas far from the estuary. It is possible that the period during which larvae or any species are available in the ocean is much longer in duration than the period over which conditions are favorable in the estuary. The window of time during which survival is highest will therefore always be occupied by larvae because larvae are available before, during, and after the window is opened. We stress the need to conduct long-term sampling programs that compare the timing and abundance of larvae on the continental shelf with the timing and abundance of larvae entering the estuary to improve our understanding of the role of local estuarine conditions in regulating species assemblage patterns.

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Effects of water temperature on protein synthesis and protein growth in juvenile Atlantic wolffish (*Anarhichas lupus*)

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Abstract: The effects of water temperature (5, 8, 11, and 14°C) on the fractional rate (percent per day) of protein consumption (k_r) and on white muscle and whole-body fractional rates of protein synthesis (k_s), protein growth (k_g), and growth efficiency (PPV, growth/consumption; k_g/k_s, growth/synthesis) of juvenile Atlantic wolffish (*Anarhichas lupus*) (initial body weight 26 g) were studied. Rates of protein consumption and white muscle and whole-body protein synthesis increased in a linear fashion between 5 and 14°C. In contrast, the relationships between temperature and white muscle and whole-body protein growth, protein growth efficiency (PPV) and protein synthesis retention efficiency (k_g/k_s) were parabolic. The results indicated that the optimum water temperatures for growth ($T_{\mathrm{opt.G}}$) and growth efficiency ($T_{\mathrm{opt.GE}}$) were 10–11 and 9–10°C, respectively. The maximum white muscle and whole-body protein growth rates recorded at $T_{\mathrm{opt.G}}$ were 0.9 and 0.7 %·day^{-1}, respectively. At $T_{\mathrm{opt.GE}}$, the maximum white muscle and whole-body PPV values were 28 and 34%, respectively, and k_g/k_s values were 92 and 51%, respectively. The growth performance data for juvenile Atlantic wolffish in comparison with published data for salmonids (rainbow trout (*Oncorhynchus mykiss*), Atlantic salmon (*Salmo salar*)) at 9–11°C further highlight its suitability as an alternative species for cold-water aquaculture in northern Europe and Atlantic Canada.

Résumé : Nous avons étudié les effets de la température de l'eau (5, 8, 11 et 14°C) sur le taux fractionnel (pour cent par jour) de la consommation de protéines (k_r) et sur les taux fractionnels de la synthèse de protéines (k_s), de la croissance protéinique (k_g) et de l'efficacité de la croissance (PPV, croissance/consommation; k_g/k_s, croissance/synthèse) dans les muscles blancs et le corps entier de loups atlantiques (*Anarhichas lupus*) (poids corporel initial de 26 g) juvéniles. Les taux de consommation de protéines et la synthèse de protéines dans les muscles blancs et le corps entier se sont accrus linéairement entre 5 et 14°C. Par ailleurs, les relations entre la température et la croissance protéinique dans les muscles blancs et le corps entier, l'efficacité de la croissance protéinique (PPV) et l'efficacité du maintien de la synthèse de protéines (k_g/k_s) étaient paraboliques. Les résultats ont indiqué que les températures de l'eau optimales pour la croissance ($T_{\mathrm{opt.G}}$) et l'efficacité de la croissance ($T_{\mathrm{opt.GE}}$) étaient de 10–11 et de 9–10°C, respectivement. Les taux de croissance protéinique maximaux dans les muscles blancs et le corps entier enregistrés à $T_{\mathrm{opt.G}}$ étaient de 0,9 et 0,7%·jour^{-1}, respectivement. À $T_{\mathrm{opt.GE}}$, les valeurs maximales de PPV dans les muscles blancs et le corps entier étaient de 28 et 34%, respectivement, et les valeurs de k_g/k_s étaient de 92 et 51%, respectivement. Les données sur la performance de la croissance chez les loups atlantiques juvéniles en comparaison des données publiées pour les salmonidés (truite arc-en-ciel (*Oncorhynchus mykiss*), saumon atlantique (*Salmo salar*)) à 9–11°C témoignent de la valeur de cette espèce à titre d'espèce de remplacement pour l'aquaculture en eau froide dans le nord de l'Europe et au Canada atlantique.

[Traduit par la Rédaction]

Introduction

In all animals, there is a continual cycle of synthesis and breakdown of protein with growth occurring under conditions where the rate of protein synthesis exceeds protein breakdown (Sugden and Fuller 1991; Houlihan et al. 1995*a*). In fish, the effects of various abiotic and nutritional influences on protein synthesis and protein growth have been studied for over 20 years and have been the subject of several reviews (Haschemeyer 1978; Fauconneau 1985; Houlihan 1991; Houlihan et al. 1993, 1995*a*, 1995*b*; McCarthy and Houlihan 1997). Water temperature has been identified as the major abiotic factor affecting the physiology and growth of fish (Brett 1979; Jobling 1997). However, although the effects of water temperature on rates of protein synthesis in fish have been well studied (reviewed by Haschemeyer 1978; McCarthy and Houlihan 1997), there are very few examples where rates of protein synthesis and growth have both been measured for the same animals (e.g., Fauconneau and Arnal 1985; Mathers et al. 1993; Reid et al. 1995,

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1997). However, these measurements are of value, as they allow the effects of water temperature on these two fundamental processes (synthesis and growth) to be modelled and also allow the effects of water temperature on the efficiency with which fish retain synthesised proteins as growth to be examined.

Our knowledge of the temperature response of protein synthesis and protein growth in fish is still limited in a number of respects. Any fish species exhibits thermal tolerance over a range of water temperatures where feeding and growth will occur and where growth performance (in terms of both rate and efficiency) can vary according to the water temperature (Jobling 1997). However, previous studies have reared groups of fish at a limited number of water temperatures within the thermal tolerance range for that species, 2°C (Reid et al. 1995, 1997) to 10°C (Fauconneau and Arnal 1985; Mathers et al. 1993) apart. To our knowledge, rates of protein synthesis and growth have not been measured over a range of water temperatures within the range of thermal tolerance for a species enabling the temperature response of these processes to be modelled. Secondly, most of the previous studies on protein synthesis and growth in fish have been carried out using salmonid fish (e.g., Fauconneau and Arnal 1985; Houlihan et al. 1986; Foster et al. 1991; Carter et al. 1993; McCarthy et al. 1994; Owen et al. 1999), most likely due to their commercial importance and the ease with which salmonids can be reared in the laboratory. It has been suggested that the pattern of protein turnover and the retention of synthesised protein as growth may vary between fish species that adopt different lifestyles (Houlihan et al. 1995*a*). For example, recent work has suggested that the proportion of synthesised proteins retained as protein growth may be higher in more sedentary fish (e.g., Atlantic halibut (*Hippoglossus hippoglossus*): Fraser et al. 1998) compared with salmonids. However, little data are available to examine this hypothesis.

The Atlantic wolffish (*Anarhichas lupus*) provides an ideal alternative model species for the study of protein metabolism in fish. It is a benthic marine fish whose habits differ from those of salmonid fish in that it has a sedentary nature and exhibits little aggression between conspecifics, factors that may promote increased growth efficiency through reduced energy expenditure. The Atlantic wolffish has also been identified as a possible candidate species for cold-water aquaculture in northern Europe (Tilseth 1990) and in Atlantic Canada (Brown et al. 1995). The progress made in the development of Atlantic wolffish aquaculture has been reviewed recently (Moksness and Pavlov 1996); however, there are no protein metabolism data available for this species. The study of the protein metabolism (rates of protein consumption, synthesis, and growth) of any potential aquaculture species will be of value in providing a better understanding of the growth performance and food conversion efficiency for that species and in allowing more meaningful comparisons to be made with the growth performance of existing culture species.

The range of thermal tolerance for the Atlantic wolffish is unknown; however, Atlantic wolffish have been recorded at water temperatures ranging between 0 and 16°C (reviewed in Moksness and Pavlov 1996). Recently, by rearing fish at a range of water temperatures between 5 and 14°C, we have shown that the optimum water temperatures for wet weight growth and growth efficiency of juvenile Atlantic wolffish are 11 and 9.6°C, respectively (McCarthy et al. 1998). At the end of that study, we had the opportunity to measure fractional rates of protein synthesis in fish sampled from each of the temperature groups, using the flooding dose method of Garlick et al. (1980) (for reviews of this technique, see Houlihan et al. 1995*a*, 1995*b*). Thus, the aims of this paper are to (*i*) examine the effect of water temperature on white muscle and whole-body rates of protein synthesis and protein growth in juvenile Atlantic wolffish, (*ii*) determine the water temperature(s) at which white muscle and whole-body protein growth rates and protein synthesis retention efficiencies are maximal, and (*iii*) compare the protein metabolism of juvenile Atlantic wolffish with that of salmonid fish (rainbow trout (*Oncorhynchus mykiss*), Atlantic salmon (*Salmo salar*)), the most commonly studied species in temperature–growth studies in fish and also the dominant fish species in cold-water aquaculture.

Materials and methods

Animal husbandry

The experiment was carried out at the Institute of Marine Research, Flødevigen Marine Research Station, Norway, between February and July 1995 using juvenile Atlantic wolffish reared from eggs obtained from adult broodstock held at the station. The origin of the broodstock and the methods of artificial fertilisation and egg incubation are described in the review of Moksness and Pavlov (1996). The fish used in this experiment were hatched and weaned onto food during May and June 1994. The holding conditions and water quality are as previously described in McCarthy et al. (1998). The thermal history experienced by these fish prior to the experiment was 6°C in May 1994, rising to about 12°C in September–October and then declining to 5°C by February 1995. This experiment was started on 20 February 1995 and lasted for 98 days. A total of 400 juvenile Atlantic wolffish were divided into four treatments, with two replicate tanks of 50 fish per treatment. Fifteen fish in each tank were individually tagged using a coding system of visible implanted fluorescent elastomer (Northwest Marine Technology, Shaw Island, WA 98286, U.S.A.) injected into their dorsal fins. A control group of 10 fish (mean weight 25.1 ± 0.7 g, $n = 10$) from the same stock were killed on 20 February 1995 in order to estimate the initial white muscle and whole-body protein content and initial white muscle fillet weight (expressed as a percentage of initial body weight) of the experimental fish. These data were used to calculate the white muscle and whole-body growth rates of the experimental fish (see below).

All groups of fish were kept in green-walled tanks (1 × 1 × 0.3 m, 260–280 L) and exposed to a 16 h light : 8 h dark photoperiod. Salinity varied between 33.6 and 34.8‰, the average oxygen saturation was about 115%, and the dissolved ammonium concentration varied between 1.0 and 6.5 μmol $NH_4 \cdot L^{-1}$. Desired experimental water temperatures were obtained using three sources: (*i*) water pumped directly from the sea channel, which fluctuated between 5 and 10°C during the study, and water from the same source that had been (*ii*) cooled to between 2 and 5°C or (*iii*) heated to between 15 and 16°C. Water from two of the three sources was mixed to provide the desired temperature of water for each tank. The water temperature in each tank was checked daily and carefully regulated to minimise day-to-day fluctuations. Nominal water temperatures of the four treatments were 5, 8, 11, and 14°C, respectively. Although the measured water temperatures varied slightly from those expected, they were not significantly different from 5, 8, 11, and 14°C (χ^2 = 1.32–3.51, 94–97 df, $p \leq 1.00$) (Table 1). The experimental groups were designated as groups 5.1, 5.2, 8.1, 8.2, 11.1, 11.2, 14.1, and 14.2, respectively.

The fish were fed a fish meal diet (NorSeaMink; Elite Plus, Skretting, Stavanger, Norway) by hand to apparent satiation two to six times per day (feeding motivation dependent on water temperature). At each feeding, the fish were fed slowly and care was taken to ensure that food offered was eaten before more was presented. The fish usually fed quickly, and uneaten food on the bottom of the tank after 5 min was taken as indicating satiation. The amount of food presented to each group was calculated by weighing the amount of food in each group's feeding jar at the beginning and end of each week.

Measurement of protein synthesis

At the end of the experiment, fractional rates of protein synthesis were measured in the surviving tagged fish in each group. On the day of injection, 24 h after their last meal, the fish were quickly weighed in a pretared beaker of water to estimate their wet weight and then injected into the peritoneum without anaesthesia with a solution containing 135 mM L-phenylalanine and L-[2,6-^{3}H]phenylalanine (Amersham International, 37 × 10^6 Bq·mL^{-1}). The injection volume administered was 1 mL·100 g body weight^{-1}, and the specific activity of the injection solution was 2682 (±117, n = 4) disintegrations per minute (dpm) per nanomole of phenylalanine. The fish were injected over 4 days in the morning (a.m.) and afternoon (p.m.) in the following order: day 95, groups 14.2 (a.m.) and 5.1 (p.m.); day 96, groups 8.1 (a.m.) and 11.1 (p.m.); day 97, groups 5.2 (a.m.) and 14.1 (p.m.); day 98, groups 11.2 (a.m.) and 8.2 (p.m.)

Following an incorporation period of about 3 h, each fish was killed by an overdose of the anaesthetic MS 222 (Sigma, Poole, Dorset, U.K), the time of death noted, and the fish weighed. The peritoneum was opened up and rinsed with water in order to remove any excess unabsorbed injection solution. For each fish, one white muscle fillet was quickly removed and weighed. The fillet and remaining carcass were frozen together on dry ice. Prior to analysis, the samples were stored at –70°C in Flødevigen and were transported from Flødevigen to Aberdeen on dry ice (total transport time <24 h). The subsequent treatment of triplicate white muscle and whole-body samples to measure the white muscle and whole-body free-pool phenylalanine-specific radioactivity (S_a), protein content, protein-bound phenylalanine-specific radioactivity (S_b), and RNA concentrations was as described in Houlihan et al. (1995*a*, 1995*b*). Values of S_a and S_b were measured using the methodologies of Suzuki and Yagi (1976) and Garlick et al. (1980). Protein contents were measured using the method of Lowry et al. (1951) as modified by Schacterle and Pollock (1973). RNA concentrations were measured using the orcinol method of Mejbaum (1939).

Calculations

The average daily fractional protein consumption rates (k_r, percent per day, expressed as a percentage of the final body protein mass) for the tagged fish were estimated as follows. The average daily protein consumption rate for each group (Con$_p$, grams protein per fish per day) was derived using the equation

$$\mathrm{Con_p} = (\mathrm{FD}\cdot 0.435)/(N_x\cdot W_{\mathrm{mid}}\cdot t)$$

where FD is the amount of feed offered to the group during the experiment, 0.435 is the proportion of protein in the diet, N_x is the average number of fish in the group ((initial + final)/2) during the experiment, W_{mid} is the average body weight of the group ((initial + final)/2), and t is the length of the experiment in days. These data are presented in table 2 of McCarthy et al. (1998). Following this, fractional rates of protein consumption (k_r, percent per day) were estimated for the tagged fish as

$$k_r = (\mathrm{Con_p}\cdot 100)/W_{\mathrm{prot}}$$

where Con$_p$ is the average daily protein consumption rate (grams protein per fish per day) and W_{prot} is the average final protein mass (i.e., wet weight × percent protein content) of the tagged fish in each group.

The white muscle and whole-body specific growth rates (SGR, percent per day) of the tagged fish were calculated according to Ricker (1979):

$$\mathrm{SGR} = [\ln(W_f) - \ln(W_0)]\cdot 100/t$$

where W_0 and W_f are the initial and final white muscle or whole-body weights of the fish, respectively, and t is the length of the experiment in days for each experimental group. A value of 38.3% (±0.5, n = 10) of the body weight, derived from the initial control group, was used to estimate the initial white muscle weight of the tagged fish. The value of t varied between experimental groups according to the days on which fish were killed: day 95 (groups 5.1 and 14.2), day 96 (groups 8.1 and 11.1), day 97 (groups 5.2 and 14.1), and day 98 (groups 8.2 and 11.2), respectively.

White muscle and whole-body fractional rates of protein synthesis (k_s, percent per day, expressed as a percentage of the protein mass synthesised per day) were calculated using the equation of Garlick et al. (1983):

$$k_s = (S_b/S_a)\cdot(1440/t)\cdot 100$$

where S_b and S_a are the protein-bound and free-pool phenylalanine-specific radioactivities and t is the incorporation time (from injection to death) in minutes. Incorporation times ranged between 169 and 208 min for individual fish with an overall average of 190 min (±8, n = 110). The mean white muscle and whole-body S_b values were 1648 dpm·nmol^{-1} (±25, n = 110) and 1615 dpm·nmol^{-1} (±39, n = 110), respectively, and attained values of 61.5% (±0.9, n = 110) and 60.2% (±1.2, n = 110) of the phenylalanine-specific radioactivity of the injection solution, respectively. White muscle and whole-body fractional protein growth rates (k_g, percent per day, expressed as a percentage of the final protein mass) were calculated using the equation of Ricker (1979) above where W_0 and W_f are the initial and final white muscle or whole-body protein masses, respectively. The initial white muscle and whole-body protein contents were estimated using the values obtained from the initial control fish (white muscle, 214.1 mg protein·g^{-1} (±7.1, n = 10); whole body, 229.8 mg protein·g^{-1} (±8.5, n = 10)). White muscle and whole-body RNA concentrations were expressed using the RNA:protein ratio, which has been termed the capacity for protein synthesis (C_s, milligrams RNA per gram protein) (Sugden and Fuller 1991). White muscle and whole-body RNA activity (k_{RNA}, grams protein synthesised per gram RNA per day) gives an approximate indication of the translational efficiency of the ribosomes and was calculated by dividing k_s by the appropriate C_s value (Houlihan et al. 1995*a*). The protein growth efficiency (PPV, percent), the efficiency with which ingested protein was retained as growth, was calculated by dividing the fractional protein growth rate by the fractional protein consumption rate. The efficiency with which synthesised proteins were retained as growth (k_g/k_s, percent) was calculated by dividing the fractional rate of protein growth by the synthesis rate.

Statistical analysis

All data are presented as mean values ± 1 SE. Two-way analysis of variance was used to (*i*) examine for differences between replicate groups at each of the four temperature treatments and (*ii*) examine the effect of water temperature on various indices of growth performance. Where the two-way analysis of variance indicated a significant temperature effect, this was further analysed using both linear (method of least squares) and quadratic regression analysis and the model of best fit (as indicated by the highest coefficient of determination, R^2) presented. Where a linear model was the best fit, analysis of covariance was used to compare the slopes of the regression lines. All statistical tests are as described in Zar (1984) and differences present at the 5% level were considered significant.

Results

The average initial body weight of the tagged fish in each of the experimental groups varied between 25.0 and 26.3 g (Table 1). Two-way analysis of variance indicated that there were no differences in the average initial body weight of the tagged fish of the two replicate groups at each temperature ($F_{(1,28)} = 1.95$, $p > 0.05$) and among the four temperature treatments ($F_{(3,84)} = 0.36$, $p > 0.05$). Likewise, at the end of the experiment, the average final body weight of the tagged fish in the two replicate groups at each water temperature were not significantly different ($F_{(1,23)} = 1.02$, $p > 0.05$). However, there was a significant temperature effect on final body weight ($F_{(3,69)} = 11.54$, $p < 0.001$). The average final body weight increased between 5 and 11°C, was highest at 11°C, and declined at 14°C (Table 1).

The effects of water temperature on white muscle specific growth rate ($\mathrm{SGR_{WM}}$) and whole-body specific growth rate ($\mathrm{SGR_{WB}}$) of the tagged fish in each group are presented in Table 1. Two-way analysis of variance indicated that for both $\mathrm{SGR_{WM}}$ and $\mathrm{SGR_{WB}}$, the growth rates of the tagged fish in the two replicate groups at each water temperature were similar ($\mathrm{SGR_{WM}}$: $F_{(1,23)} = 0.51$, $p > 0.05$; $\mathrm{SGR_{WB}}$: $F_{(1,23)} = 0.97$, $p > 0.05$) and there was a significant temperature effect ($\mathrm{SGR_{WM}}$: $F_{(3,69)} = 12.87$, $p < 0.001$; $\mathrm{SGR_{WB}}$: $F_{(3,69)} = 7.76$, $p < 0.001$). Both $\mathrm{SGR_{WM}}$ and $\mathrm{SGR_{WB}}$ exhibited a similar parabolic relationship to water temperature, increasing between 5 and 11°C and declining at 14°C with growth rates being highest at 11°C (Table 1), and were best described by the following equations:

$$\mathrm{SGR_{WM}} = -0.826 + 0.330 \cdot T - 0.016 \cdot T^2$$
$$(R^2 = 0.733,\ n = 8,\ p < 0.02)$$

$$\mathrm{SGR_{WB}} = -0.690 + 0.293 \cdot T - 0.014 \cdot T^2$$
$$(R^2 = 0.761,\ n = 8,\ p < 0.01)$$

where T is the water temperature (degrees Celsius).

Differentiation of the regression coefficients indicated that maximum $\mathrm{SGR_{WM}}$ and $\mathrm{SGR_{WB}}$ values were at 10.3 and 10.5°C, respectively. As expected, the fish exhibited isometric growth: least squares regression analysis of the relationships between $\mathrm{SGR_{WB}}$ and $\mathrm{SGR_{WM}}$ at the four water temperatures indicated slope coefficients ranging between 1.04 and 1.07 (data not shown).

The effects of water temperature on the fractional rate of protein consumption (k_r) and whole-body and white muscle fractional rates of protein synthesis (WB k_s and WM k_s) and protein growth (WB k_g and WM k_g) are presented in Fig. 1. Values of k_r increased with increasing water temperature between 5 and 14°C (Fig. 1*a*), the relationship of which was best described by a linear model (see caption to Fig. 1*a*). For each of WB k_s, WM k_s, WB k_g, and WM k_g, two-way analysis of variance indicated that there were no differences between the average fractional rates of the tagged fish in the two replicate groups at each water temperature ($F_{(1,23)} = 0.89$ (WB k_s), 0.18 (WM k_s), 1.68 (WB k_g), and 1.72 (WM k_g), respectively, all $p > 0.05$), but there was a significant temperature effect (WB k_s: $F_{(3,69)} = 2.71$, $p < 0.05$; WM k_s: $F_{(3,69)} = 2.95$, $p < 0.05$; WB k_g: $F_{(3,69)} = 18.70$, $p < 0.001$; WM k_g: $F_{(3,69)} = 9.55$, $p < 0.001$). WB k_s (Fig. 1*b*) and WM k_s (Fig. 1*c*) increased in a linear fashion with increasing water temperature. Analysis of covariance indicated that there was no difference between the slopes of the two regression lines ($t = 1.09$, 12 df, $p > 0.05$), indicating a common temperature response in the fractional rates of protein synthesis in the whole-body and white muscle (Figs. 1*b* and 1*c*). The relationships between water temperature and WB k_g and WM k_g were parabolic; fractional protein growth rates increased between 5 and 11°C, were highest at 11°C, and decreased at 14°C (Figs. 1*d* and 1*e*). Differentiation of the regression coefficients for these relationships (see captions to Figs. 1*d* and 1*e*, respectively) indicated that maximum WB k_g and WM k_g values were at 11.2 and 10.2°C, respectively. The percent protein contents of the white muscle ($\mathrm{PRO_{WM}}$) and whole body ($\mathrm{PRO_{WB}}$) are shown in Table 1. Two-way analysis of variance indicated that for both $\mathrm{PRO_{WM}}$ and $\mathrm{PRO_{WB}}$, the percent protein contents of the tagged fish in the two replicate groups at each water temperature were similar ($\mathrm{PRO_{WM}}$: $F_{(1,23)} = 3.35$, $p > 0.05$; $\mathrm{PRO_{WB}}$: $F_{(1,23)} = 2.73$, $p > 0.05$). $\mathrm{PRO_{WM}}$ increased with increasing water temperature ($F_{(3,69)} = 3.08$, $p < 0.05$), although no temperature effect was observed with $\mathrm{PRO_{WB}}$ ($F_{(3,69)} = 2.39$, $p > 0.05$).

The relationships between water temperature and protein growth efficiency in the whole body ($\mathrm{PPV_{WB}}$) and white muscle ($\mathrm{PPV_{WM}}$) and between water temperature and the efficiency of retention of synthesised proteins in the whole body (WB k_g/k_s) and white muscle (WM k_g/k_s) were parabolic and are shown in Fig. 2. $\mathrm{PPV_{WB}}$ values increased between 5 and 11°C, were maximal at 11°C, and decreased at 14°C (Fig. 2*a*). $\mathrm{PPV_{WM}}$ values also appeared to be exhibit a parabolic relationship to water temperature (Fig. 2*b*), although this relationship was not significant at the 5% level ($p < 0.07$). Differentiation of the regression coefficients for these relationships (see captions to Figs. 2*a* and 2*b*, respectively) indicated that maximum $\mathrm{PPV_{WB}}$ and $\mathrm{PPV_{WM}}$ values were at 10.0 and 9.5°C, respectively. Two-way analysis of variance indicated that for both the WB k_g/k_s and WM k_g/k_s values, there were no differences between the replicate groups at each water temperature (WB k_g/k_s: $F_{(1,23)} = 0.92$, $p > 0.05$; WM k_g/k_s: $F_{(1,23)} = 1.42$, $p > 0.05$), but there was a significant temperature effect (WB k_g/k_s: $F_{(3,69)} = 4.72$, $p < 0.01$; WM k_g/k_s: $F_{(3,69)} = 2.86$, $p < 0.05$). Both WB k_g/k_s and WM k_g/k_s values increased between 5 and 11°C, were maximal at 11°C, and decreased at 14°C (Figs. 2*c* and 2*d*). Differentiation of the regression coefficients for these relationships (see captions to Figs. 2*c* and 2*d*, respectively) indicated that maximum WB k_g/k_s and WM k_g/k_s values were at 10.4 and 8.6°C, respectively.

The effects of water temperature on the whole-body and white muscle capacity for protein synthesis (WB C_s and WM C_s) and ribosomal translational efficiency (WB k_{RNA} and WM k_{RNA}) are shown in Fig. 3. For each of WB C_s, WM C_s, WB k_{RNA}, and WM k_{RNA}, two-way analysis of variance indicated that there were no differences between the average values of the tagged fish in the two replicate groups

Table 1. Initial and final numbers (N_0 and N_f), initial and final body weights (W_0 and W_f, g), white muscle (SGR_{WM}) and whole-body (SGR_{WB}) specific growth rates (%·day^{-1}), and white muscle (PRO_{WM}) and whole-body (PRO_{WB}) protein contents (% wet weight) of juvenile Atlantic wolffish (*Anarhichas lupus*) at 5, 8, 11, and 14°C.

Group	Temperature (°C)	N_0	W_0	N_f	W_f	SGR_{WM}	SGR_{WB}	PRO_{WM}	PRO_{WB}
5.1	4.9 (0.04)	15	26.3 (0.6)	15	46.0 (2.0)	0.39 (0.06)	0.43 (0.05)	21.1 (1.0)	21.4 (0.8)
5.2	4.8 (0.04)	15	25.3 (0.8)	15	40.8 (1.9)	0.54 (0.05)	0.48 (0.04)	18.9 (0.9)	19.5 (0.9)
8.1	7.7 (0.08)	15	26.7 (0.6)	11	48.8 (3.8)	0.68 (0.09)	0.62 (0.08)	18.4 (0.7)	19.7 (0.9)
8.2	7.8 (0.05)	15	25.8 (0.7)	14	56.3 (3.6)	0.74 (0.09)	0.73 (0.07)	21.4 (1.0)	22.3 (1.0)
11.1	10.9 (0.08)	15	25.5. (0.7)	14	64.6 (5.7)	0.98 (0.10)	0.94 (0.09)	19.2 (1.1)	19.8 (0.6)
11.2	11.0 (0.07)	15	25.8 (0.5)	14	64.9 (2.9)	1.03 (0.05)	0.93 (0.05)	22.9 (1.2)	22.7 (0.8)
14.1	14.0 (0.07)	15	26.3 (1.0)	12	52.5 (3.9)	0.70 (0.12)	0.65 (0.10)	22.7 (1.3)	21.0 (0.9)
14.2	13.9 (0.05)	15	25.0 (0.5)	15	50.1 (5.1)	0.67 (0.12)	0.66 (0.10)	22.1 (0.9)	21.9 (0.5)

Note: Values are presented as mean ± 1 SE.

at each water temperature ($F_{(1,23)} = 0.31$ (WB C_s), 0.10 (WM C_s), 0.76 (WB k_{RNA}), and 0.22 (WM k_{RNA}), respectively, all $p > 0.05$), but there was a significant temperature effect (WB C_s: $F_{(3,69)} = 12.09$, $p < 0.001$; WM C_s: $F_{(3,69)} = 14.63$, $p < 0.001$; WB k_{RNA}: $F_{(3,69)} = 16.23$, $p < 0.001$; WM k_{RNA}, $F_{(3,69)} = 15.02$, $p < 0.001$). The relationships between water temperature and WB C_s and WM C_s were linear with the capacity for protein synthesis decreasing with increasing water temperature (Fig. 3*a*). WB k_{RNA} and WM k_{RNA} values exhibited an exponential increase with increasing water temperature (Fig. 3*b*) and, following log transformation of the k_{RNA} values, were best described by a linear regression model (see caption to Fig. 3*b*). Analysis of covariance indicated that there was no difference between the slopes of the two regression lines ($t = 1.27$, 12 df, $p > 0.05$), indicating a common temperature response in RNA activity in the whole body and white muscle.

Discussion

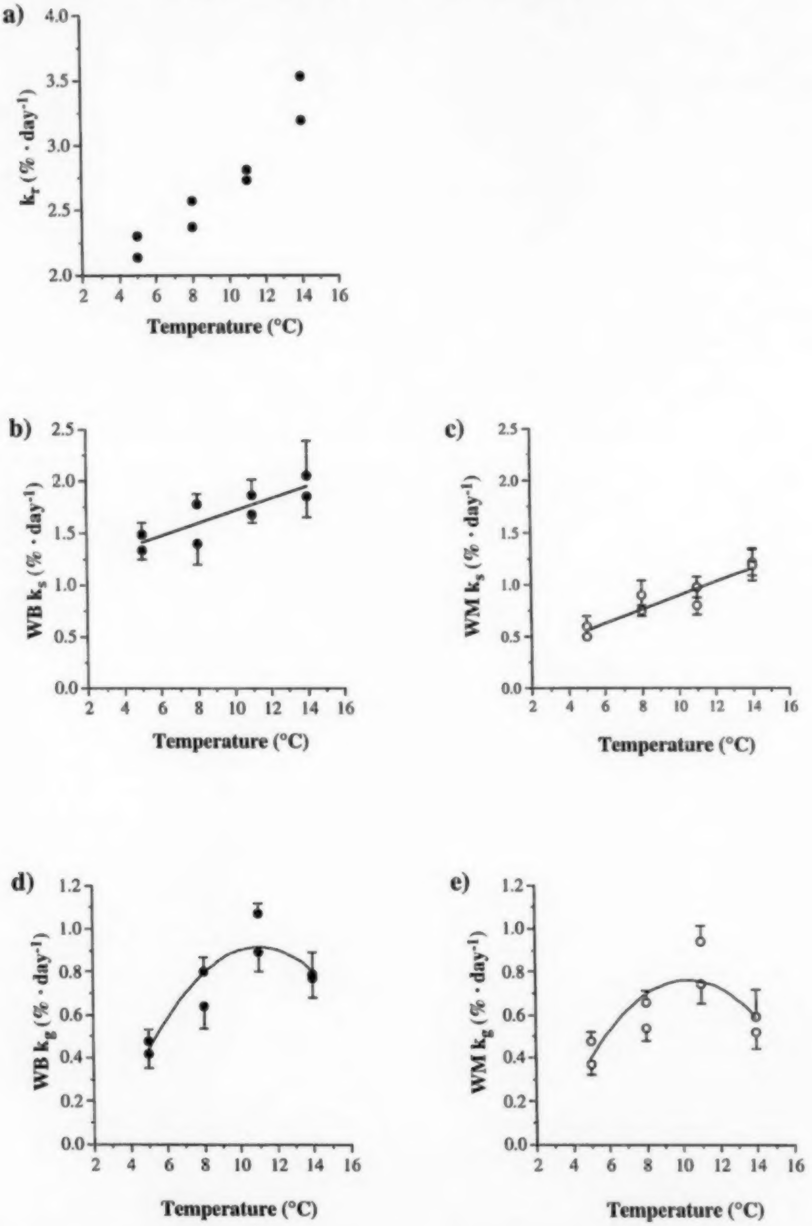
For ectotherms, the relationship between the rate of many biochemical or physiological processes and water temperature is asymmetrical. Providing that resources are not limiting and the temperature increase is within the thermal limits for the species, the rate of any biochemical or physiological process will increase with increasing water temperature to a maximum, above which the rate declines near the upper limit for the species (Huey and Kingsolver 1989; Atkinson 1994). Since growth performance is the net expression of physiological performance, these parameters would also be expected to follow an asymmetrical relationship with temperature. In fish, consumption rates, growth rates, and growth efficiency (growth/consumption) all follow this temperature-dependent increase to an optimum followed by a decline with any further increase in water temperature. Previous studies have shown that the optimum water temperature for consumption ($T_{opt.R}$) is higher than the optimum water temperature for growth ($T_{opt.G}$), which in turn is higher than the optimum water temperature for growth efficiency ($T_{opt.GE}$) (Woiwode and Adelman 1991; Imsland et al. 1995; Björnsson and Tryggvadóttir 1996; Jobling 1997). In this study, the protein consumption rates, growth rates, and growth efficiency of juvenile Atlantic wolffish were examined at 5, 8, 11, and 14°C. Protein consumption rates increased over this range of water temperatures, and it was not possible to identify $T_{opt.R}$ (Fig. 1*a*) (also see McCarthy et al. 1998). However, the growth rate and growth efficiency data of the tagged juvenile Atlantic wolffish did exhibit a temperature-dependent pattern, and it was possible to determine $T_{opt.G}$ and $T_{opt.GE}$. The results of this study indicated that $T_{opt.G}$ for whole-body and white muscle specific growth rates and fractional protein growth rates in juvenile Atlantic wolffish was 10–11°C (Table 1; Fig. 1) and that $T_{opt.GE}$ for protein growth efficiency and protein synthesis retention efficiency in both the whole body and white muscle of juvenile Atlantic wolffish was 9–10°C (Fig. 2). As expected, these values are in agreement with the $T_{opt.G}$ and $T_{opt.GE}$ values for whole-body specific growth and growth efficiency (wet weight gain/dry weight of food offered) obtained using the group growth rate data from this experiment (McCarthy et al. 1998).

This is the first study to measure fractional rates of protein synthesis in juvenile Atlantic wolffish. Therefore, it is desirable to examine whether the protein synthesis rates measured at 5, 8, 11, and 14°C in this study are within the range of values to be expected for an ectotherm at these water temperatures. In a recent review on the effects of water temperature on protein synthesis in fish, McCarthy and Houlihan (1997) summarised the general effect of water temperature on the process of muscle protein synthesis using the available ectotherm and endotherm data. Figure 4 summarises the relationship between temperature and muscle protein synthesis derived from these data (references are cited in McCarthy and Houlihan (1997) or are available from the corresponding author). The fractional rate of muscle protein synthesis (k_s) increased exponentially with increasing temperature (T°C) and, following logarithmic transformation of the protein synthesis values, was best described by the following linear regression equation:

$$\log_{10} k_s = 0.035T°\mathrm{C} - 0.529 \qquad (R^2 = 0.738,\ n = 34,\ p < 0.001).$$

The average fractional rates of white muscle protein synthesis for juvenile Atlantic wolffish at 5, 8, 11, and 14°C measured in this study (0.55, 0.82, 0.88, and 1.20%·day^{-1}, respectively) are also shown in Fig. 4. These average values were significantly higher than the muscle protein synthesis

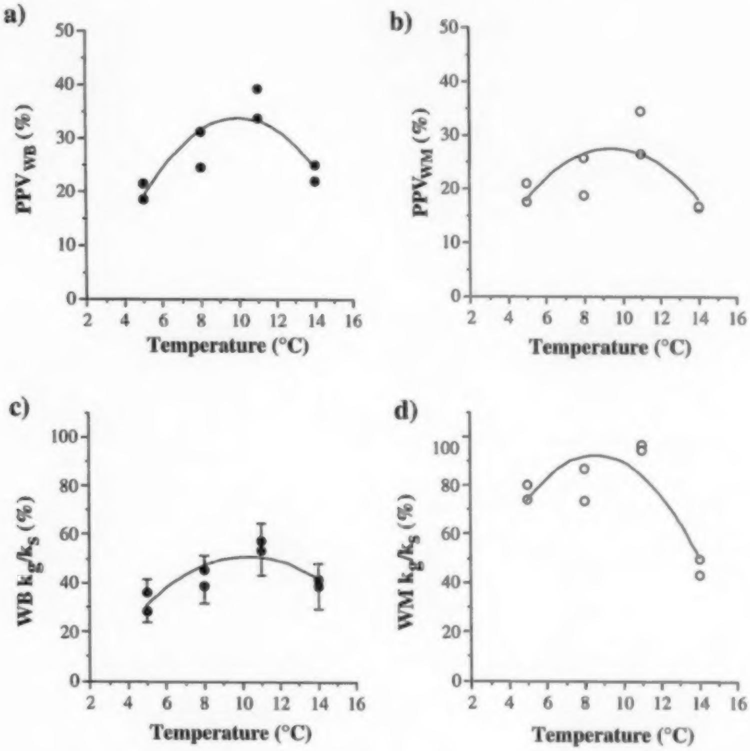
Fig. 1. Relationships between water temperature (*T*) and (*a*) the fractional rate of protein consumption, k_r ($k_r = 1.527 + 0.124 \cdot T$; $R^2 = 0.881$, $n = 8$, $p < 0.001$), (*b*) the fractional rate of whole-body protein synthesis, WB k_s (WB $k_s = 1.105 + 0.060 \cdot T$; $R^2 = 0.693$, $n = 8$, $p < 0.006$), (*c*) the fractional rate of white muscle protein synthesis, WM k_s (WM $k_s = 0.230 + 0.067 \cdot T$; $R^2 = 0.854$, $n = 8$, $p < 0.001$), (*d*) the fractional rate of whole-body protein growth, WB k_g (WB $k_g = -0.695 + 0.290 \cdot T - 0.013 \cdot T^2$; $R^2 = 0.774$, $n = 8$, $p < 0.01$), and (*e*) the fractional rate of white muscle protein growth, WM k_g (WM $k_g = -0.604 + 0.264 \cdot T - 0.013 \cdot T^2$; $R^2 = 0.652$, $n = 8$, $p < 0.03$), for juvenile Atlantic wolffish (*Anarhichas lupus*). Values are presented as mean ± 1 SEM.



rates predicted from the regression analysis (0.44, 0.56, 0.72, and 0.91%·day^{-1}, respectively) (paired *t* test, $t = 4.87$, 3 df, $p < 0.02$) but were not significantly different from the muscle protein synthesis rates predicted at the upper 95% confidence limit for the above regression line at 5, 8, 11, and 14°C (0.59, 0.74, 0.93, and 1.18%·day^{-1}, respectively) (paired *t* test, $t = 0.08$, 3 df, $p > 0.05$).

In this study, under satiation feeding conditions, white muscle and whole-body fractional rates of protein synthesis increased with water temperature between 5 and 14°C

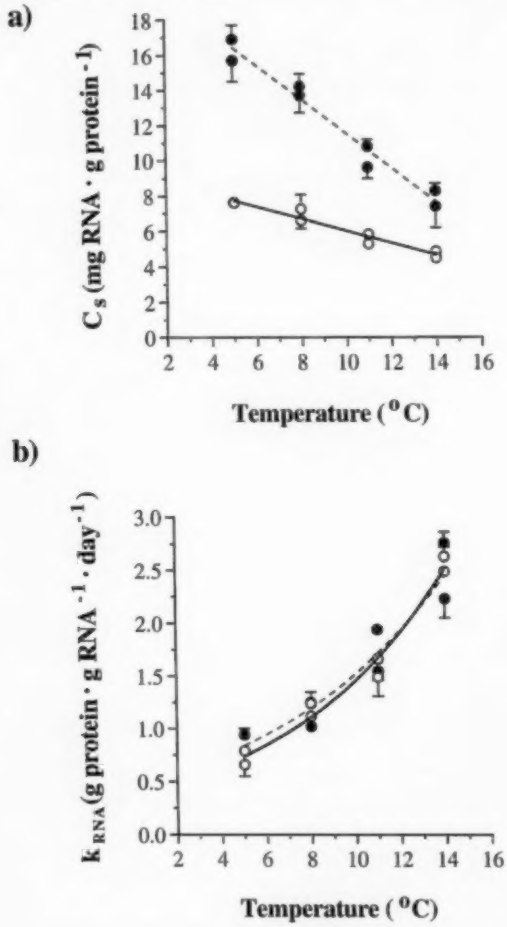
Fig. 2. Relationships between water temperature (T) and (a) whole-body protein growth efficiency, PPV_{WB} ($PPV_{WB} = -25.314 + 11.720 \cdot T - 0.583 \cdot T^2$; $R^2 = 0.609$, $n = 8$, $p < 0.04$), (b) white muscle protein growth efficiency, PPV_{WM} ($PPV_{WM} = -15.191 + 8.933 \cdot T - 0.469 \cdot T^2$; $R^2 = 0.503$, $n = 8$, $p < 0.07$), (c) whole-body protein synthesis retention efficiency, WB k_g/k_s (WB $k_g/k_s = -24.110 + 14.410 \cdot T - 0.693 \cdot T^2$; $R^2 = 0.614$, $n = 8$, $p < 0.04$), and (d) white muscle protein synthesis retention efficiency, WM k_g/k_s (WM $k_g/k_s = -14.792 + 24.778 \cdot T - 1.438 \cdot T^2$; $R^2 = 0.617$, $n = 8$, $p < 0.04$), for juvenile Atlantic wolffish (*Anarhichas lupus*). Values are presented as mean ± 1 SEM.



(Figs. 1*b* and 1*c*). These results are in agreement with earlier studies that have shown that protein synthesis rates in fish increase with increasing water temperature when food is not limiting (Fauconneau and Arnal 1985; Loughna and Goldspink 1985; Watt et al. 1988; Mathers et al. 1993; McCarthy and Houlihan 1997) compared with no increase in synthesis rates when food is restricted (Foster et al. 1992). In this study, between 5 and 14°C, a linear model was the best descriptor of the relationship between water temperature and protein synthesis in juvenile Atlantic wolffish (Figs. 1*b* and 1*c*). However, there is evidence to suggest that the relationship between protein synthesis and water temperature is asymmetrical with maximum rates of protein synthesis occurring at the optimum temperature for any single species. Pannevis and Houlihan (1992) measured in vivo rates of protein synthesis in isolated hepatocytes from rainbow trout at incubation temperatures of 5, 10, 14.5, 17.5, and 20°C and reported an asymmetrical relationship between protein synthesis and temperature (their fig. 2) with the highest rates occurring at 14.5 and 17.5°C, close to the $T_{opt.G}$ for rainbow trout of 17°C (Hokanson et al. 1977). The work of Loughna and Goldspink (1985) also suggests the possibility of maximum rates of protein synthesis occurring at the $T_{opt.G}$ for common carp (*Cyprinus carpio*) and rainbow trout. Their results suggest that the relationship between protein synthesis and water temperature may reach an asymptote at water temperatures of 25 and 15–20°C for common carp and rainbow trout, respectively (their fig. 2). These temperature values are close to the $T_{opt.G}$ values that have previously been reported for these species, 27°C for common carp (Goolish and Adelman 1984) and 17°C for rainbow trout (Hokanson et al. 1977), and would support the hypothesis that maximum rates of protein synthesis occur at water temperatures close to the $T_{opt.G}$ value for a particular species. To date, however, where the effect of water temperature on in vivo rates of protein synthesis has been measured on whole animals (as opposed to isolated cell cultures) over a range of water temperatures (Loughna and Goldspink 1985; this study), the range selected for study has not included a sufficient number of post-$T_{opt.G}$ temperature values to allow this hypothesis to be tested

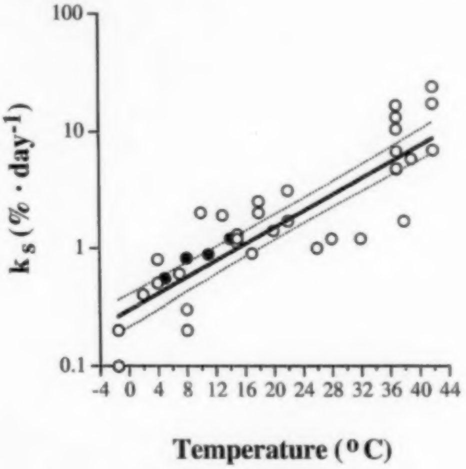
In this study, the capacity for protein synthesis (C_s, milligrams RNA per gram protein) in the white muscle and whole body increased with decreasing water temperature (Fig. 3*a*). This increase in RNA concentration with decreasing water temperature has been noted in previous studies (e.g., Goolish et al. 1984; Foster et al. 1992; Mathers et al. 1993) and is

Fig. 3. Relationships between water temperature (T) and (a) whole-body (WB, solid circles) and white muscle (WM, open circles) RNA to protein ratios, C_s (WB $C_s = 21.292 - 0.970 \cdot T$; $R^2 = 0.963$, $n = 8$, $p < 0.001$; WM $C_s = 9.437 - 0.341 \cdot T$; $R^2 = 0.939$, $n = 8$, $p < 0.001$), and (b) whole-body (WB, solid circles) and white muscle (WM, open circles) k_{RNA} values ($\log_{10}$(WB k_{RNA}) $= -0.340 + 0.052 \cdot T$; $R^2 = 0.922$, $n = 8$, $p < 0.001$; $\log_{10}$(WM k_{RNA}) $= -0.428 + 0.059 \cdot T$; $R^2 = 0.970$, $n = 8$, $p < 0.001$) for juvenile Atlantic wolffish (*Anarhichas lupus*). Values are presented as mean ± 1 SEM.



recognised as a thermal compensatory response by fish at colder water temperatures where the rate at which proteins are translated during the ribosome cycle is lower (McCarthy and Houlihan 1997). This decrease in translation rates at low water temperatures is also indicated by the decrease in white muscle and whole-body k_{RNA} values (grams protein synthesised per gram RNA per day), also known as "RNA activity" or "RNA translational efficiency" (Sugden and Fuller 1991), observed in this study (Fig. 3b). This decrease in k_{RNA} with decreasing temperature has been observed in both single-species studies (e.g., Foster et al. 1992; this study) and is also a general biological phenomenon observed across ectothermic and endothermic vertebrate species (McCarthy and Houlihan 1997, their fig. 3b).

Fig. 4. Relationship between temperature and muscle fractional rates of protein synthesis, k_s (open circles), for ectotherms (fish, frog, lizard) and endotherms (birds, mammals) (note logarithmic y-axis). The regression line (solid line) and its 95% confidence limits (dotted lines) are also presented. The least squares linear regression analysis of the data, following logarithmic transformation of the protein synthesis rates, is presented in the text. The sources of these data are cited in McCarthy and Houlihan (1997) and are also available from the corresponding author. The average white muscle k_s values at 5, 8, 11, and 14°C for the juvenile Atlantic wolffish in this study are indicated by the solid circles.



In this study, although whole-body fractional rates of protein synthesis were higher than those in the white muscle (Figs. 1b and 1c), both exhibited a similar response to water temperature as indicated by the similar slope coefficients (see captions to Figs. 1b and 1c, respectively). McCarthy and Houlihan (1997) have also shown a common temperature response when comparing the general effect of temperature on protein synthesis in the liver and muscle of ectotherms and endotherms (their fig. 3a). Despite significant differences in relative rates at any given temperature, protein synthesis rates being higher in the liver compared with the muscle, similar slope coefficients were observed for the two tissues. However, in contrast with the protein synthesis results, liver and muscle k_{RNA} values did not differ and there was a single temperature response (McCarthy and Houlihan 1997, their fig. 3b): this single temperature response was also seen in the whole-body and white muscle k_{RNA} values calculated in this study (Fig. 3b). At any given temperature, RNA concentrations are known to vary significantly between different tissues, when expressed on a weight-specific basis (milligrams RNA per gram) or as the capacity for protein synthesis (C_s, milligrams RNA per gram protein), in both ectotherms (e.g., Fauconneau and Arnal 1985; Foster et al. 1991; Martin et al. 1993) and endotherms (e.g., Garlick et al. 1980, 1983; Sugden and Fuller 1991). Therefore, it appears that tissue-specific differences in RNA concentrations, rather than tissue-specific differences in RNA translational efficiency, are responsible for the differences in relative synthesis rates observed between tissues. However, both tissue and whole-body fractional protein synthesis rates and tissue and

Table 2. Comparison between the fractional protein growth rate (k_g, %·day^{-1}) and protein synthesis retention efficiency (k_g/k_s, %) in the white muscle (WM) and whole body (WB) of juvenile Atlantic wolffish and salmonid fish.

Species	Body weight (g)	Temperature (°C)		k_g	k_g/k_s	Reference
Atlantic wolffish (*Anarhichas lupus*)	65	9–11	WB	0.7	51	This study
			WM	0.9	92	This study
Rainbow trout (*Oncorhynchus mykiss*)	65	10	WB	0.9	35	McCarthy et al. (1994)
			WM	1.0	67	I.D. McCarthy et al. (unpublished)
	65	12	WM	0.4[a]	73[a]	Houlihan et al. (1986)
	75	12	WB	2.0	45	Foster et al. (1991)
	108	10	WB	0.9	30	Fauconneau and Arnal (1985)
			WM	1.3	52	Fauconneau and Arnal (1985)
Atlantic salmon (*Salmo salar*)	37	12	WB	1.6	53	Owen et al. (1999)

Note: The body weight and temperature for each study are also presented.

[a] Calculated from equations presented in table 2 of Houlihan et al. (1986).

whole-body k_{RNA} values appear to follow a common temperature response as indicated by similar slope coefficients. Also, for both protein synthesis and k_{RNA}, a single line for both ectotherms and endotherms indicates a commonality in these processes controlled simply by temperature.

The study of protein synthesis and protein growth is of particular relevance to aquaculture of finfish species, where the aim in culture is essentially to maximise white muscle protein deposition. As a result, much of the work on protein synthesis and growth in fish has been carried out on salmonids (e.g., Fauconneau and Arnal 1985; Houlihan et al. 1986; Carter et al. 1993; McCarthy et al. 1994; Owen et al. 1999), as these are the dominant culture species in cold-water aquaculture. The suitability of any new finfish species for cold-water aquaculture will be, in part, dependent on its growth performance, in terms of growth rate and growth efficiency, in comparison with salmonids. Therefore, the results obtained for Atlantic wolffish in this study were compared with those of salmonid fish of a comparable size at a similar water temperature. Table 2 provides a comparison of the whole-body and white muscle fractional protein growth rates and protein synthesis retention efficiencies for juvenile Atlantic wolffish at 9–11°C (i.e., the range covering their $T_{opt.G}$ and $T_{opt.GE}$ values) with values obtained for salmonid fish of a comparable size (40–100 g) at a similar water temperature (10–12°C). The whole-body and white muscle fractional protein growth rates of juvenile Atlantic wolffish recorded in this study were lower than most of the growth rate values for salmonid fish of a comparable size presented in Table 2. The growth rate of juvenile Atlantic wolffish is significantly influenced by factors such as feed composition, dietary protein source, and the physical characteristics of the pellets (floating or sinking) (reviewed in Moksness and Pavlov 1996; McCarthy et al. 1998). However, increased growth rates will be possible as the optimal dietary formulation and pellet design for juvenile Atlantic wolffish become known and rearing conditions are optimised (McCarthy et al. 1998). The protein synthesis retention efficiencies for juvenile Atlantic wolffish recorded in this study were higher for both the whole body (51% cf. 35–53%) and white muscle (92% cf. 52–73%) compared with salmonid fish at a similar water temperature. This increased growth efficiency, particularly in the white muscle, may be due to a reduction in energy expenditure as a result of the behaviour of wolffish under culture conditions. In comparison with salmonid fish, Atlantic wolffish exhibit much less swimming activity with both juveniles and adults spending long periods of time inactive on the tank bottom (reviewed in Moksness and Pavlov 1996). Also, the only phase of the life cycle where any overt aggression has been observed is as small juveniles, 50–100 mm in length. However, in comparison with salmonid fish, the frequency of aggression is much lower (reviewed in Moksness and Pavlov 1996) and no consequent body injuries have been reported to date. This reduction in aggression will both lower the energy costs associated with activity and reduce the negative effects of stress on intermediary metabolism (Van der Boon et al. 1991) in Atlantic wolffish. The growth performance results of this study further highlight the potential of Atlantic wolffish as an alternative species for cold-water aquaculture in northern Europe and Atlantic Canada.

Houlihan et al. (1995*a*) have suggested that the pattern of protein turnover and protein retention efficiency may vary between fish species that differ in their lifestyle. For example, herbivorous fish appear to be able to better utilise a wider range of diets, exhibiting higher rates of protein synthesis and protein growth and increased protein retention efficiencies, compared with carnivorous fish (Houlihan et al. 1995*a*). The results of this study also suggest that differences in protein metabolism may exist between sedentary and active fish species. Whole-body protein synthesis retention efficiencies (k_g/k_s) of 51–77% have been recorded for sedentary fish species such as Atlantic cod (*Gadus morhua*) (53%, Houlihan et al. 1995*a*), Atlantic wolffish (51%, this study), and Atlantic halibut (77%, Fraser et al. 1998). In comparison, whole-body protein synthesis retention efficiencies of 30–53% have been reported for the more active salmonid fish (Table 2). Also, this study has shown a higher white muscle protein synthesis retention efficiency in a sedentary species, 92% for Atlantic wolffish, compared with values of 52–73% for more active salmonids. This increased efficiency may be a result of lower turnover rates of muscle myofibrillar proteins in a less active fish. However, to test this hypothesis, a comparative study of myofibrillar growth and turnover rates in a sedentary and active fish species is necessary.

Acknowledgements

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Decadal changes in growth and recruitment of Pacific halibut (*Hippoglossus stenolepis*)

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Abstract: Since the climate regime shift of 1976–1977 in the North Pacific, the individual growth of Pacific halibut (*Hippoglossus stenolepis*) has decreased dramatically in Alaska but not in British Columbia. Recruitment has increased dramatically in both areas. The decrease in age-specific vulnerability to commercial longline gear resulted in a persistent underestimation of incoming recruitment by the age-structured assessment method (CAGEAN) that was used to assess the stock. This problem has been corrected by adding temporal trends in growth and fishery selectivity to the assessment model. The recent sustained high level of recruitment at high levels of spawning biomass has erased the previous appearance of strong density dependence in the stock–recruitment relationship and prompted a reduction in the target full-recruitment harvest rate from 30–35 to 20–25%. The climate regime shift affected a number of other stocks of vertebrates and invertebrates in the North Pacific. While the general oceanographic changes have now been identified, the specific biological mechanisms responsible for the observed changes have not.

Résumé : Depuis le changement du régime climatique de 1976–1977 dans le Pacifique Nord, la croissance individuelle du flétan du Pacifique (*Hippoglossus stenolepsis*) a diminué de façon marquée en Alaska, mais non en Colombie-Britannique. Le recrutement s'est accru de façon marquée dans les deux régions. La réduction de la vulnérabilité selon l'âge à la pêche commerciale à la palangre a eu pour résultat une sous-estimation persistante du recrutement par la méthode d'évaluation structurée en fonction de l'âge (CAGEAN) qui a été utilisée pour évaluer le stock. On a corrigé ce problème en intégrant au modèle d'évaluation des tendances temporelles de la croissance et de la sélectivité de la pêche. Le récent niveau élevé soutenu de recrutement associé à des niveaux élevés de biomasse de reproducteurs a effacé la forte dépendance apparente passée de la relation stock–recrutement à l'égard de la densité et a entraîné une réduction du taux de récolte cible à plein recrutement de 30–35 à 20–25%. Le changement du régime climatique a eu un impact sur divers autres stocks de vertébrés et d'invertébrés dans le Pacifique Nord. Tandis que les changements océanographiques généraux sont aujourd'hui connus, les mécanismes biologiques spécifiques responsables des modifications observées ne le sont pas.

[Traduit par la Rédaction]

Introduction

The Pacific halibut (*Hippoglossus stenolepis*) is widely distributed in coastal waters of the North Pacific from central California to the northern Bering Sea and on the Asian side south to Hokkaido. Aboriginal peoples in North America have fished halibut for thousands of years, and a commercial longline fishery has been conducted in U.S. and Canadian waters for more than 100 years. Since 1923 the stock has been studied and managed by the International Pacific Halibut Commission (IPHC), making this one of the longest- and best-studied groundfish stocks in the world. Halibut assessment and management have been described by Skud (1977*a*), Hoag et al. (1993), and Sullivan and McCaughran (1995).

During this century, there have been dramatic and persistent changes in the growth and recruitment of halibut that cannot be explained by changes in stock size. Growth was slow early in the century, rapid in the middle years, and again slow in the most recent years. The growth changes were much larger in Alaska than in British Columbia. In the case of recruitment, the pattern of alternation between periods of high and low recruitment was often represented as a cycle of about 20 years (e.g., Parma and Deriso 1990), although there was no known reason for this cyclic behavior.

Recent climatological research has provided a likely explanation of these changes as direct or indirect results of abrupt changes in the climate of the North Pacific. These changes, called "regime shifts", occur every few decades, the most recent event having occurred in the winter of 1976–1977. This coincides closely with the recent downturn in halibut growth rates and the most recent upsurge in halibut recruitment and with pronounced changes in the distribution and abundance of other species (Francis et al. 1998).

At this point, it is clear that decadal changes in the climate of the North Pacific cause decadal changes in the life history parameters and productivity of the halibut stock and stocks of other species. It is not at all clear what mechanisms are at work. In this paper, we review the nature and timing of the changes that we have observed in the halibut

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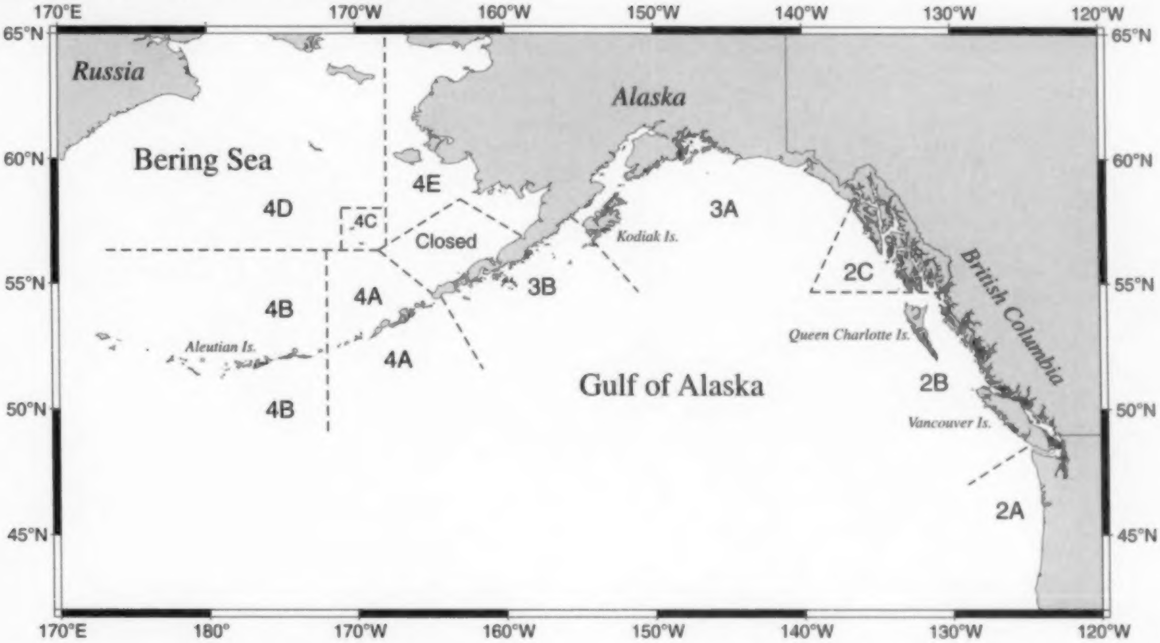
Fig. 1. IPHC regulatory areas in the Northeast Pacific.



stock and the effect of these changes on the way that IPHC assesses and manages the stock. We also briefly summarize the current understanding of regime shifts in the North Pacific and discuss alternative hypotheses concerning the effects of climate and other factors on the halibut stock.

Changes in growth schedule and other life history parameters

Halibut on the American side of the North Pacific (Fig. 1) spawn in midwinter in the Gulf of Alaska and Bering Sea. After about 6 months in the plankton, the postlarvae settle out and metamorphose mostly in the western Gulf of Alaska and southeastern Bering Sea. Some of the juveniles from these nursery grounds migrate west and south to populate the central and eastern Gulf of Alaska. By age 8 the migration is complete and movement thereafter is minimal except for a seasonal spawning migration from a summer feeding area on the continental shelf to a winter spawning area in deeper water on the continental slope (Skud 1977*b*). The halibut fishery is conducted mainly in spring and summer when the fish are on their summer feeding grounds.

Owing to the size selectivity of longline gear, hardly any fish below a length of about 60 cm are caught in either survey or commercial operations. This length corresponds approximately to an age of 6 years. The commercial fishery seeks out larger fish and in addition has been subject to a minimum size limit of 32 in. (81 cm) since 1974, so the youngest age-class to appear in any numbers in the commercial landings is age 8. The modal age in the commercial landings is usually 11 or 12 years, and nearly all of the landings consist of fish 8–20 years old. Females are substantially larger than males (on average) at each age. Fish are eviscerated at sea, so the sex of the fish in the commercial landings cannot be determined.

The most useful data on the growth of halibut come from systematic longline surveys that have been conducted by IPHC from time to time since the mid-1960s, mostly in northern British Columbia (IPHC Area 2B) and around Kodiak Island (IPHC Area 3A) (see Fig. 1). Additional useful data are available from research charters conducted for other purposes in 1988 and 1989. The average size-at-age in survey catches is, of course, affected by the size selectivity of longline gear, but changes over time in average size-at-age in survey catches reflect changes in growth. The sex of all the fish in survey catches is recorded, so the effect of sexual dimorphism can be excluded.

Between the mid-1970s and the mid-1990s, the growth of halibut in Alaska decreased dramatically (Fig. 2, top panels). A similar but smaller change occurred in British Columbia (Fig. 2, bottom panels). In the mid-1970s, halibut grew faster and larger in Alaska than in British Columbia; at present, there is hardly any difference.

Data from research cruises in the first half of this century indicate conditions in the 1920s and 1930s very similar to those at present, with much lower growth rates than in the middle of the century and no difference in size-at-age between Alaska and British Columbia. These earlier data are not strictly comparable with recent setline survey data, in that they were collected on tagging trips in the winter rather than on systematic surveys in the summer. Nevertheless, the changes in both data series are so large that there is no doubt that the growth pattern of halibut in the 1920s and 1930s was very similar to the present schedule.

A detailed examination of the growth paths of individual year-classes of females in the Kodiak region over the last 20 years shows no sign of an abrupt change at the time of

Fig. 2. Mean length-at-age by sex and period of Pacific halibut caught in setline surveys in the northern Gulf of Alaska (IPHC Area 3A) and British Columbia (IPHC Area 2B).

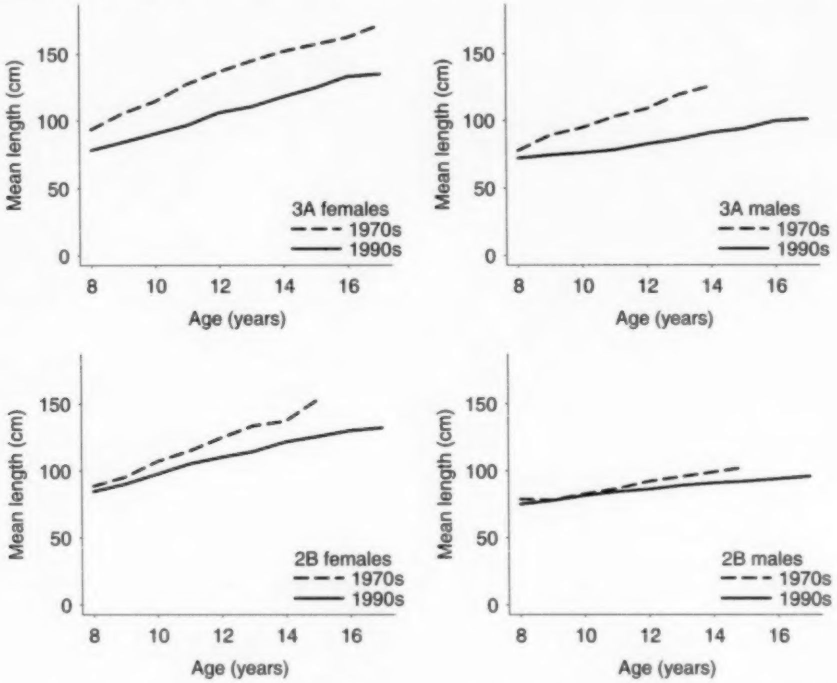
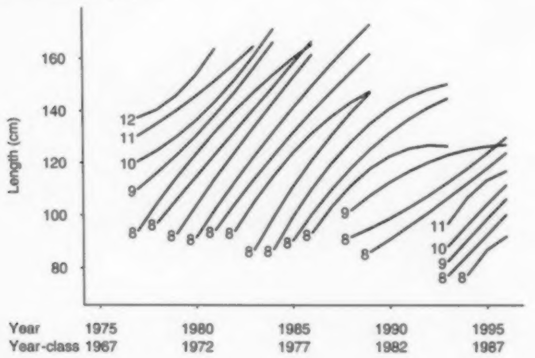


Fig. 3. Growth paths by year-class of female Pacific halibut caught in setline surveys in IPHC Area 3A. The paths plotted are quadratics fitted to the length-at-age data for each year-class. The numbers at the beginning of the solid lines are the age at which the year-class first appeared in a survey. Surveys were not conducted every year.



the 1977 climate change. Growth does not appear to have decreased substantially until the middle or late 1980s (Fig. 3), but then decreased drastically at all ages. The most recent years' data indicate some recovery in growth rates among fish older than age 8, but the incoming 8-year-olds remain much smaller than in earlier years.

The age at which 50% of females have reached sexual maturity changed little during this period, remaining at 11–12 years in both Alaska and British Columbia. However, because of the decline in growth rates, this resulted in a large decrease in the length at 50% maturity from 110–125 cm in the 1970s to 90–100 cm now (Fig. 4).

Effect of growth changes on the IPHC stock assessment

During the 1980s and into the 1990s, the IPHC staff estimated halibut stock size annually using the method of catch-at-age analysis called CAGEAN, described by Deriso et al. (1985). This procedure consists of fitting a separable model in which fishing mortality at each age in each year is assumed to be the product of a year-specific full-recruitment fishing mortality rate and an age-specific selectivity (partial recruitment factor). The schedule of age-specific selectivities is assumed to be constant over all the years of catch-at-age and catch-per-effort data to which the model is fitted.

The vulnerability of halibut to longline gear increases with the size of the fish over a considerable range of sizes, and there is a minimum commercial size limit of 32 in. (81 cm), so it could be expected that a decrease in size-at-age would alter the age-specific selectivity of the commercial fishery and introduce some bias into the annual stock assessment. It did.

The bias first became apparent in a retrospective examination of biomass estimates in the early 1990s, in which current estimates of biomass in a given year were compared with previous estimates of biomass in that year. Retrospective application of the CAGEAN procedure to a moving 15-year window of data, simulating the sequence of annual

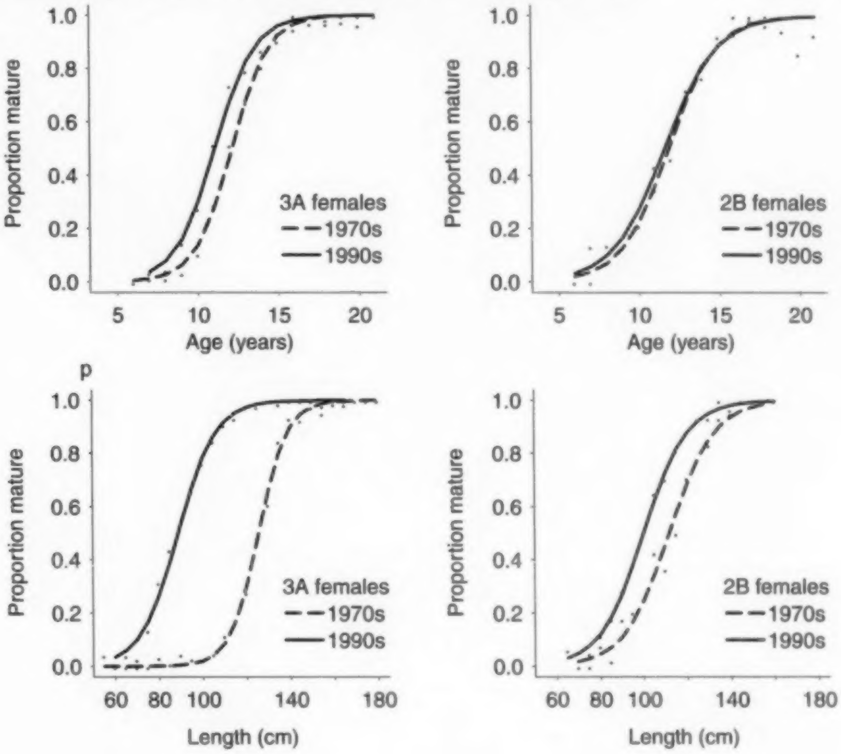


Fig. 4. Maturity as a function of age (top panels) and length (bottom panels) of female Pacific halibut caught in setline surveys in the northern Gulf of Alaska (IPHC Area 3A) and British Columbia (IPHC Area 2B) by period.

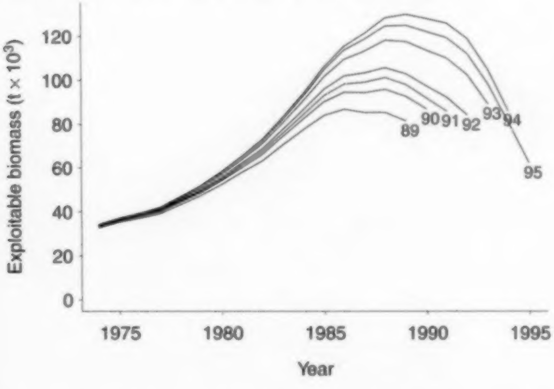


Fig. 5. Retrospective pattern of CAGEAN Pacific halibut estimates in IPHC Area 3A. Note how the estimate of biomass in 1989 increases as more years of data are added.

assessments with no changes in the model, showed that up to the mid-1980s the initial estimate of biomass in a given year was substantially higher than the estimates obtained in subsequent assessments based on more recent data (Parma 1993). Then the pattern reversed, so that during the late 1980s and early 1990s the initial estimates were substantially lower than the subsequent estimates based on more recent data (Fig. 5). During this latter period the standard stock assessment showed a steady decline in stock size and a drastic decline in recruitment.

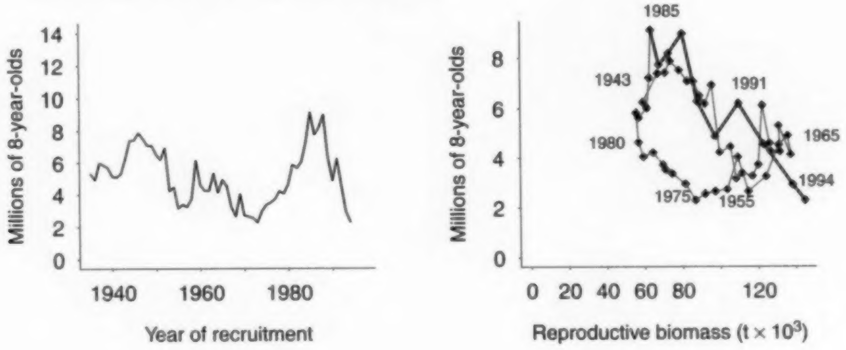
Retrospective patterns usually result from trends in parameters that are assumed to be constant in the model. In this case the age-specific selectivities were suspect because of the ongoing decline in size-at-age in the landings, but they were not the only suspect. The model was tuned to commercial catch-at-age and commercial catch-per-effort, and fishing practices were changing immediately before and after the implementation of individual quota management in Canada (1991) and Alaska (1995).

Until 1986, IPHC had conducted systematic setline surveys to provide fishery-independent data on trends in abundance, growth, and maturity. These surveys were resumed in 1993, and they confirmed the trends seen in the commercial fishery. In Alaska the survey catch rates closely matched the increasing trend of commercial catch rates and in British Columbia indicated an even larger increase. The decline in size-at-age was seen to be a real change for both females and males, rather than an artifact of changed fishing practices or a changed sex ratio.

To account for the effects of the decline in size-at-age, the staff developed a new model that treats selectivity as size specific rather than age specific and allows for changes over time in the growth schedule, with resulting changes over time in the implied age-specific selectivities (Sullivan et al. 1999). Where CAGEAN estimated year-class strengths,

Fig. 6. Recruitment trends and stock–recruitment relationship of Pacific halibut in the Northeast Pacific estimated using CAGEAN, a separable catch-at-age model used in past assessments, and the new assessment model in which selectivity changes as a function of size and age.

Estimates obtained with CAGEAN



Estimates obtained with new model

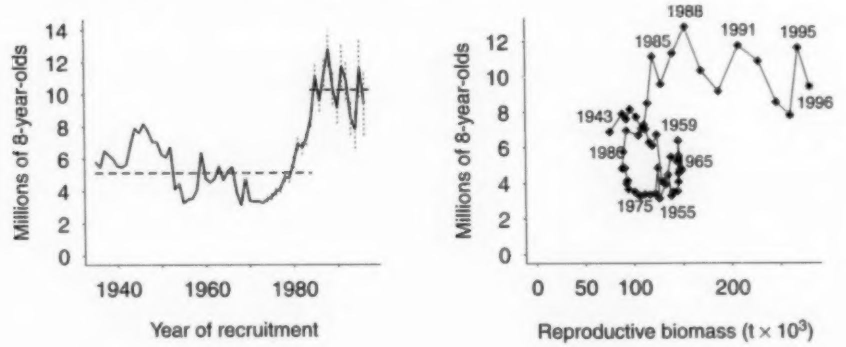
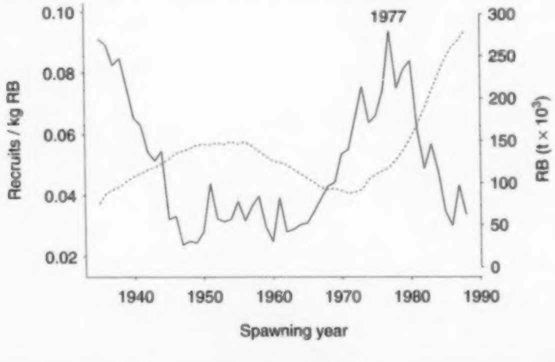


Fig. 7. Trends in reproductive biomass (RB) (broken line) and recruits per unit of reproductive biomass (solid line).



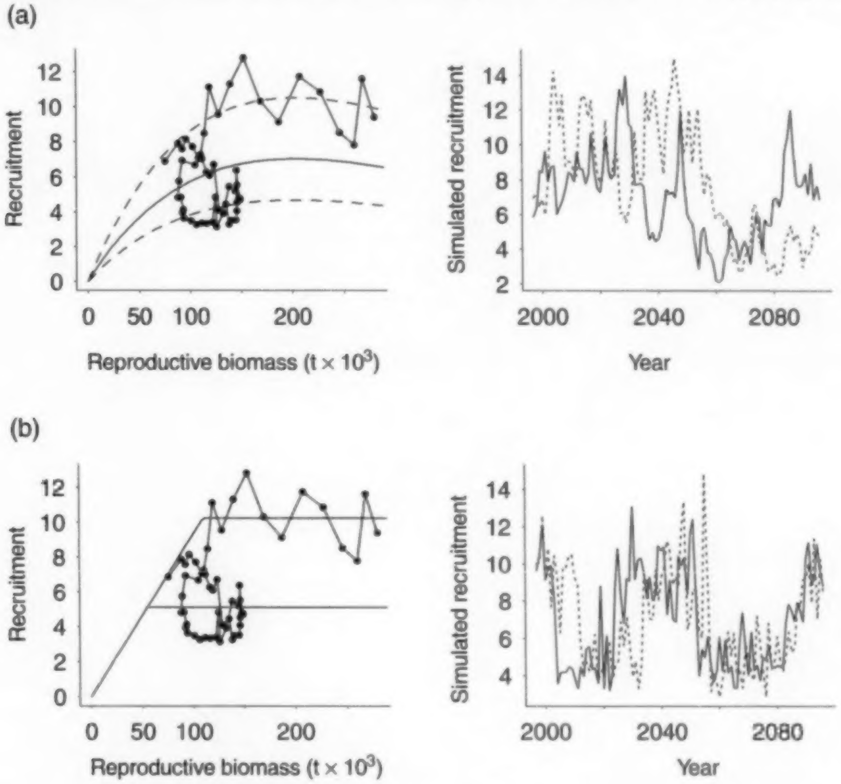
year-specific (full-recruitment) fishing mortality rates, and constant age-specific selectivities, the new model estimates year-class strengths, year-specific fishing mortality rates, time-varying growth parameters, and size-specific selectivities for both the survey and the commercial fishery.

We initially assumed that size-specific selectivities in the survey (not the commercial fishery) had remained constant over time, and we fitted the model with a constant size-specific survey catchability and selectivity to survey and commercial catch rates, age compositions, and size-at-age distributions. Size-specific commercial catchability and selectivity were allowed to change over time, so the fits were controlled mainly by the survey data.

The fits were puzzling. IPHC uses exactly the same survey design, gear, and fishing schedule in British Columbia and Alaska, yet the model fits showed very different size-specific survey selectivities in the two areas. Survey selectivity increased from nil at 60 cm to full vulnerability at 90 cm in British Columbia, but not until 120 cm in Alaska. This suggested that selectivity was not simply a function of the gear and the growth schedule, but likely depended on some distributional or behavioral features that were more closely related to age than to size.

The size-specific model can be made to serve as an age-specific model by allowing the survey size-specific selectivities to change over time, like the commercial selectivities, but imposing a heavy penalty on any variation over time in the implied age-specific survey selectivities. This requires the survey selectivities to change over time as

Fig. 8. Alternative recruitment models used to evaluate harvest policies. The right-hand panels show examples of simulated trajectories. (*a*) Ricker model with autocorrelated environmental effects; (*b*) flat model with shifts in carrying capacity.



size-at-age changes in such a way that the implied age-specific survey selectivities remain effectively constant.

Fitting the model with the assumption of constant age-specific (rather than size-specific) survey selectivity produced similar estimates of the general trend of stock biomass in British Columbia and Alaska over the last 20 years, although the estimates of year-class strength in Alaska in recent years were substantially lower. The age-specific model fit largely eliminated the retrospective pattern that CAGEAN showed in Alaska, but not in British Columbia.

Both models produced estimates of present exploitable biomass more than double the previous CAGEAN estimates. In British Columbia, where the change in size-at-age was modest, the increase resulted mainly from fitting to survey rather than commercial catch rates. Survey catch rates in British Columbia in the 1990s indicated a substantially larger increase since the mid-1980s then did commercial catch rates. In Alaska, where the trends in commercial catch rates closely matched survey results but the change in size-at-age was dramatic, the increase resulted mainly from relaxing the assumption of a constant age-specific commercial selectivity. Recent recruitment, which had appeared to be plummeting, now appears to be soaring (Fig. 6).

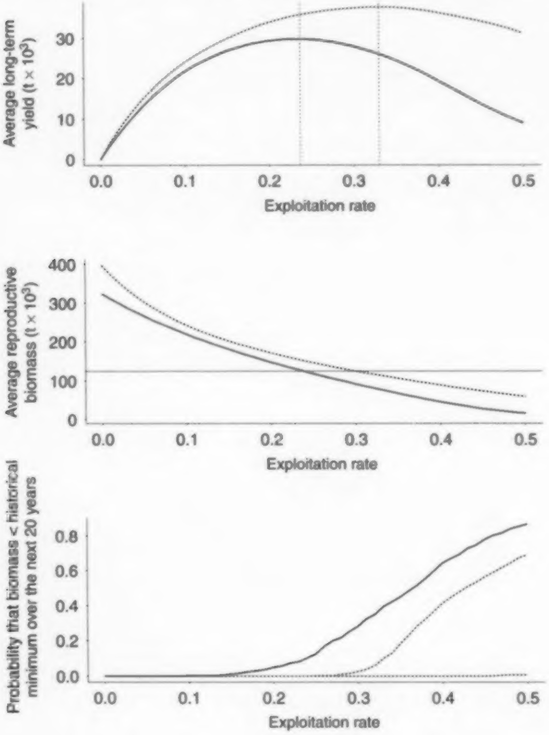
At this point, we cannot say which model is more appropriate. The staff's quota recommendations for 1997 were based on the age-specific model mainly because the biomass estimates were a little lower and we preferred to err on the low side. It is somewhat ironic that with all the information about the halibut stock that is available to us, dating back to the 1920s, we are unsure of our assessment because we do not know how to interpret the survey data. In a few years, of course, when the recently recruited year-classes in Alaska have passed through the fishery, we will be better able to estimate their size and in turn find out which assumption about survey selectivity is more likely to be true.

Changes in recruitment and harvest strategy

The dramatic decline in recruitment of 8-year-olds estimated by CAGEAN during the late 1980s and 1990s coincided with a period of increasing spawning biomass during the early 1980s (Fig. 6, top panels). Opposite trends in the number of 8-year-olds and parental biomass gave support to the hypothesis that the stock–recruitment relationship was strongly density dependent exhibiting overcompensation.

The new stock assessment results show a very different relationship between spawning biomass and subsequent recruitment in recent years. Instead of declining, recruitment estimates for the last 10 years either fluctuate without clear trend (Fig. 6, bottom panels) or increase, depending on whether survey selectivity is assumed to be a function of age or size,

Fig. 9. Performance of different exploitation rates under two different stock–recruitment models, a Ricker model with autocorrelated environmental effects (solid line) and a flat model with shifts in carrying capacity (broken line). The vertical lines indicate the yield-maximizing harvest rates for the two models.



respectively. In either case, recruitment appears to have fluctuated independently of stock size, at least over the observed range of spawning biomass levels.

The assessment results indicate that the number of recruits produced per unit of reproductive biomass (Fig. 7) has changed by a factor of 4, showing persistent periods of low and high productivity. Peaks in productivity occur during periods of both low and high parental stock abundance and so cannot be readily explained by changes in parental biomass.

Recruitment levels estimated for 1985–1996 (year-class 1977 and subsequent) under the most conservative assumption (age-dependent selectivity) are on average about twice the average recruitment level estimated for the preceding 40 years. While the estimates for the last few years are particularly uncertain (see confidence intervals in Fig. 6), the timing of the increase in recruitment coincides with major changes in climatic regime across the North Pacific (discussed below).

IPHC sets the total allowable removals of halibut by applying a constant harvest rate to the estimate of exploitable biomass. The harvest rate is chosen by simulating stock productivity at a range of harvest rates under the operation of a range of spawner–recruit relationships consistent with historical estimates of spawning biomass and subsequent recruitment. In the past, both Beverton–Holt and Ricker curves were fitted to the estimates obtained with CAGEAN, and they implied harvest rates of 0.30–0.35 to obtain maximum yield.

The recruitment estimates obtained with the new models do not indicate strong density dependence. They do indicate that the environment has played a major role in driving recruitment variation, at least within the range of stock levels observed. With this new view of historical recruitment patterns, we have considered a number of new stock–recruitment relationships for evaluating alternative harvest strategies. In all of the relationships explored, a great deal of the recruitment variability is induced by the environment and so is not under management control. Two are described here.

(1) A Ricker (1954) model with autocorrelated environmental effects (Fig. 8*a*). The number of recruits at age 8 is given by

$$R_{t+8} = S_t \cdot \exp(a - bS_t + \varepsilon_t)$$

where S_t is reproductive biomass and the series of $\{\varepsilon_t\}$ represent environmental effects. The latter are modeled as an autoregressive process of order 1:

$$\varepsilon_t = \rho\varepsilon_{t-1} + e_t$$

where e_t is a normal random variable with mean 0 and variance σ^2. The parameter ρ corresponds to the correlation between ε_t and ε_{t-1}. Parameters a, b, ρ, and σ^2 were fitted by maximum likelihood to present estimates of historical spawning biomass and subsequent recruitment. Two sample recruitment trajectories simulated with this model are shown in Fig. 8*a*.

(2) A flat model with shifts in carrying capacity (Fig. 8*b*). In this scenario, expected recruitment increases in proportion to reproductive biomass until carrying capacity (K_i) is reached and is constant thereafter:

$$R_{t+8} = \min(aS_t, K_i) \cdot \exp(\varepsilon_t)$$

Carrying capacity is affected by environmental conditions that shift randomly and irregularly between two very different regimes every 20–30 years so that K_i alternates between two values, K_1 and K_2. There is additional process noise represented by the series of normally distributed random variables $\{\varepsilon_t\}$. The slope parameter a of the flat model was set at the maximum value of R_{t+8}/S_t in the assessment results. The alternative carrying capacities K_1 and K_2 were set to the antilogarithms of the mean log recruitments in the periods 1943–1984 and 1985–1996, respectively.

Both models predict that recruitment will decrease gradually as spawning biomass decreases to levels lower than the historical minimum. Predictions made about how juvenile production would change if the stock dropped to unprecedented stock levels are extremely uncertain, as they are based on an extrapolation beyond the range of historical experience.

Alternative exploitation rates were evaluated by simulating how the stock would respond to different harvest rates ranging from 0.0 to 0.50 under the operation of the new stock–recruitment relationships and with the new, slower growth schedule. A standard age-structured model was used to simulate future trends in abundance and catches under the different harvesting regimes. Recruitment was generated according to one of the stock–recruitment models described above. Weights-at-age were assumed to be fixed at the values estimated from the catch of 1996, so the possibility of future trends in growth was ignored.

Stock trajectories were simulated for 200 years, beginning at the estimated present stock level (plus or minus some random assessment error), and the performance of alternative harvest rates was evaluated on the basis of their average performance during the last 100 years of each of 500 replicates. Measures of performance were mean long-term yield, mean level of reproductive biomass, and the probability that the reproductive biomass dropped below 75 000 t (round weight), approximately the historical minimum (attained in the mid-1930s and again in the mid-1970s), over the first 20 years of simulation. While the exploitation rate that resulted in maximum long-term yield differed for the two models considered (0.23 compared with 0.33), a range of harvest rates between 0.20 and 0.30 resulted in average yields that were within 10% of the respective maxima in both cases (Fig. 9). Even under a very optimistic scenario in which recruitment remains at the high level indicated by the last 11 estimates (i.e., a flat stock–recruitment model with carrying capacity stable at the most productive level), yields produced under a 0.25 harvest rate were only 17% lower than the maximum possible yield (obtained with a harvest rate close to 0.50).

Long-term average reproductive biomass for harvest rates between 0.20 and 0.25 ranged between 120 000 and 160 000 t, well above the historical minimum (Fig. 9). Minimum levels of reproductive biomass attained in the simulated trajectories depend on the stock–recruitment model. Under the Ricker model with autocorrelated environmental effects, the probability that the stock dropped below the historical minimum over the first 20 years of simulation was small (<10%) for harvest rates of 0.20 and lower, but it increased substantially when the harvest rate was raised above 0.25 (Fig. 9, bottom panel). The probability of dropping below the historical minimum increased more slowly for harvest rates exceeding 0.30 under the flat model, as the simulated carrying capacity could remain high for several years before switching back to the low level. If, instead, high recruitment levels were assumed for the next 20 years, this probability was close to zero. These two cases are shown by the broken lines in Fig. 9 (bottom panel).

Although it is impossible to predict long-term average yield levels, as these will depend on future environmental conditions, results indicate that harvest rates ranging from 0.20 to 0.30 may achieve close-to-maximum yields under different hypotheses about future stock productivity (Fig. 9, top panel). Harvest rates in the range 0.20–0.25 may achieve close-to-maximum yields under different recruitment scenarios while having a high probability that the stock level stays within the range of historical abundance.

Concurrent climatic and ecosystem changes in the Northeast Pacific

The changes in Pacific halibut growth and recruitment that we have related took place in a biological and climatic setting that has also undergone decadal scale variability this century. The nature of this variability has been characterized as abrupt shifts between different states, or regimes. The most recent regime shift in the North Pacific occurred in the winter of 1976–1977 (Miller et al. 1994). Several investigators have speculated that the 1976–1977 regime shift is but the most recent in a succession of events (Francis and Hare 1994; Hare and Francis 1995; Ware 1995; Hare 1996; Mantua et al. 1997; Minobe 1997; Francis et al. 1998). In the twentieth century, it has been proposed that four distinct climatic regimes have occurred. The regimes have averaged 25–30 years in duration, with the transitions taking place in the mid-1920s, mid-1940s, and mid-1970s. This climatic phenomenon has recently been termed the Pacific Decadal Oscillation (Mantua et al. 1997).

Biological changes associated (or at least coincident) with these climatic regime shifts have been spectacular. Evidence of biophysical connections are evident throughout the North Pacific and Bering Sea ranging from the plankton to the highest trophic levels. Abrupt changes in population size following the winter of 1976–1977 have been documented for primary production (Venrick et al. 1987), zooplankton (Brodeur and Ware 1992), crustaceans (Otto 1990; Anderson 1991), and marine birds and mammals (Piatt and Anderson 1996; Merrick et al. 1997). Alaska salmon populations have shown perhaps the best documented response to North Pacific climate regime shifts (Beamish and Bouillon 1993; Francis and Hare 1994; Bigler et al. 1996). An extensive summary of North Pacific ecosystem response to interdecadal climate variability is contained in Francis et al. (1998).

Many species of demersal and pelagic fish experienced exceptionally strong year-classes from 1976 to 1978 (Beamish 1993). High survival apparently occurred in response to improved ocean productivity associated with changes in the intensity of the Aleutian Low. Strong year-classes and increased productivity occurred all along the Northeast Pacific from California into the Bering Sea. Beamish (1993) speculated that the 1976–1977 climate event influenced productivity in the California Current through changes in upwelling and in the North Pacific through climate changes.

Hollowed and Wooster (1992, 1995) evaluated recruitment patterns and ocean conditions over a longer time period, primarily from the 1950s into the 1980s. They identified synchronous strong year-classes of Northeast Pacific groundfish and attempted to associate environmental conditions with the strong year-classes. Alternating warm and cool periods of sea surface temperature, averaging about a decade in duration, have occurred at least since 1932. These periods are much more frequent than the regime shifts that occur on a decadal scale. Synchronous strong recruitment occurred during a warm period. Not all warm periods exhibit synchronous strong year-classes, and the species that contribute strong year-classes vary from period to period. Warm periods may be necessary, but not sufficient, for strong year-classes (Hollowed and Wooster 1995).

Northeast Pacific groundfish show varied patterns of abundance over the period for which data are available (Bakkala 1993; North Pacific Fishery Management Council 1996*a*, 1996*b*). Major species in the Bering Sea and Gulf of Alaska showed a synchronous increase in abundance from the mid-1970s to the mid-1980s. Subsequently, patterns changed. Walleye pollock (*Theragra chalcogramma*), the most abun-

Fig. 10. Abundance trends of (*a*) arrowtooth flounder in the Gulf of Alaska and (*b*) rock sole in the Bering Sea.

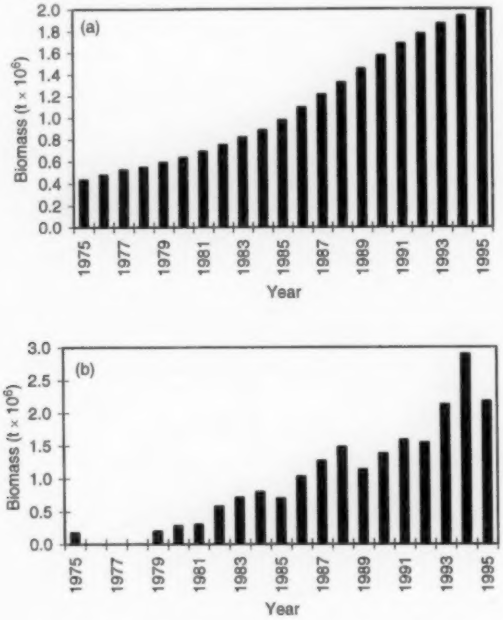
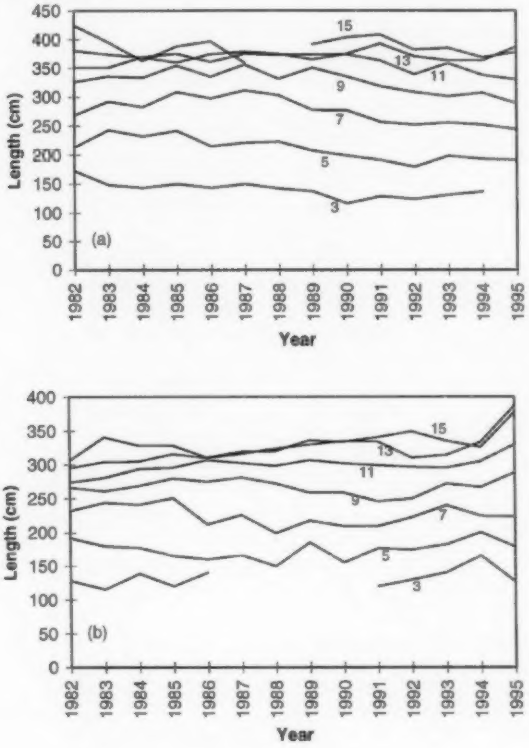


Fig. 11. Length-at-age of (*a*) rock sole and (*b*) yellowfin sole in the Bering Sea.



dant species in the Northeast Pacific, is currently decreasing from the mid-1980s peak. Sablefish (*Anoplopoma fimbria*) in the Gulf of Alaska and Bering Sea – Aleutian Islands have decreased slightly (Gulf of Alaska) to greatly (Bering Sea). Pacific cod (*Gadus macrocephalus*) initially declined from the late 1980s, but a strong peak in abundance occurred around 1994.

Most major flatfish species in the Gulf of Alaska and the Bering Sea increased in concert with other groundfish from the mid-1970s to the mid-1980s, but either remained at high levels (Bering Sea yellowfin sole (*Pleuronectes asper*)) or continued to increase into the early 1990s (Gulf of Alaska arrowtooth flounder (*Atheresthes stomias*), Bering Sea rock sole (*Pleuronectes bilineatus*)) (Fig. 10). Like Pacific halibut, Bering Sea rock sole and yellowfin sole and Gulf of Alaska arrowtooth flounder experienced very strong recruitment in the 1980s and into the 1990s relative to recruitment in the 1960s and early 1970s. Yellowfin sole, unique among the flatfish, experienced serious overfishing during the early 1960s but showed the same recruitment and abundance trends as most other flatfish. In contrast with halibut and other flatfish, Greenland halibut (*Reinhardtius hippoglossoides*) peaked in spawning biomass during the mid-1970s and recruitment was high through the middle and late 1970s. Since then, spawning biomass has declined steadily to the current level of about 15–20% of the peak value and about a third of the earliest record (1970). Recruitment has fluctuated at low levels during the 1980s and 1990s.

Length-at-age for Bering Sea yellowfin sole and rock sole, two flatfish with abundance fluctuations similar to those of Pacific halibut, show little of the changes evident for halibut length-at-age (Alaska Fisheries Science Center, National Marine Fisheries Service, Seattle, Wash., unpublished data). Neither species showed any change over the 1982–1995 period of data availability (Fig. 11), while halibut growth rates dropped sharply in the late 1980s.

Discussion

The dramatic changes that we have observed in the growth and recruitment of Pacific halibut are clearly just one consequence of the most recent regime shift in the North Pacific, which has affected all components of the ecosystem directly and indirectly. One would not expect that all species, with differing life history strategies, ranges, and adaptations, would have responded similarly to the regime shift of 1976–1977. It is surprising, however, how pervasive the changes were at all trophic levels. Many, perhaps most, of the major populations of the Northeast Pacific underwent major changes, some up and some down, following that winter.

Decadal shifts in population parameters have complicated the assessment of the halibut stock. As explained above, the change in growth and maturity schedules may or may not have altered the age-specific selectivity of IPHC setline surveys used for tuning the assessment. We will find out within a few years, and then we should be able to model survey selectivity correctly over time.

Regime shifts pose less of a problem for management, at least for the choice of a harvest strategy. A constant harvest rate strategy turns out to be very robust to changes in carrying capacity (Walters and Parma 1996), and as shown above, it is possible to locate a harvest rate that performs well across a family of spawner–recruit curves of widely varying resiliency (i.e., slope at the origin). The worst problem in the area of policy evaluation is the common confounding of environmental and density-dependent effects. Environmentally driven trends in recruitment can easily appear as spurious evidence of density dependence (Parma and Deriso 1990) and lead to a poor choice of harvest rate. Nonstationarity in the stock–recruitment relationship should always be considered a possibility when evaluating alternative harvest strategies, even in the absence of clear signs of environmental effects.

While fixed harvest rate strategies have been shown to cope well with nonstationary stock–recruitment relationships, their performance may deteriorate when climatic shifts induce changes in growth as well, as in the case of Pacific halibut. Changes in size and maturity-at-age can strongly affect the lifetime reproductive contribution per recruit, which may require compensatory adjustments to the target harvest rate.

The most difficult challenge posed by decadal climate shifts is determining the specific biological mechanisms that produce the observed changes in a particular stock like Pacific halibut. In the case of growth, for example, the observed large reduction in growth in Alaska (and the much smaller reduction in British Columbia) could have been a direct result of higher temperatures in Alaska. Temperature has been shown to explain most of the regional and temporal variation observed in Atlantic cod (*Gadus morhua*) stocks in the North Atlantic (Brander 1995; Campana et al. 1995). The general decrease in salmon weight-at-age and decreased size-at-maturity has been attributed, directly and indirectly, to warmer temperatures in the Gulf of Alaska (Hinch et al. 1995; Cox and Hinch 1997). Other plausible causes of reduced halibut growth are intraspecific competition due to increased halibut abundance and interspecific competition with other burgeoning flatfish populations. Since all these changes occurred at the same time, it is hard to identify what effect each had.

The performance of other flatfish stocks in Alaska argues against a strong effect of interspecific competition. While halibut growth rates have declined since the mid-1970s, the growth rates of rock sole and yellowfin sole in Alaska have not. This suggests that causes other than simple food supply are responsible for the observed growth changes. A change in the quality of prey may have occurred. While we are a long way from determining a cause for the changes in halibut growth rates, the mechanism must be consistent with increased survival of individuals, yet a slower growth for those individuals, similar to what was observed among Alaska salmon stocks.

The climate regime shift has, to date, been described in terms of processes acting at the atmosphere–ocean interface. Constructing reasonable linkages between atmospheric and oceanic processes and surface-dwelling (plankton, salmon) or surface-dependent (birds, mammals) animals is relatively straightforward. The link to groundfish, however, is more indirect and more difficult to investigate. Compared with the ocean's surface, very little is known about the variability of conditions at the ocean's bottom above the continental shelf and slope where groundfish reside. It is by no means certain that conditions at depth mirror those at the surface.

A number of climatically forced bottom-up and top-down mechanisms can be hypothesized to explain the observed changes in halibut biology. Teasing apart the various influences will require creative investigation. Nevertheless, there is reason for optimism. The 60+-year halibut otolith record provides a longer time series for analysis than is available for most other species. In addition, there are lengthy catch, recruitment, and growth time series. Progress in the physical sciences has resulted in more comprehensive environmental databases. More sophisticated modeling and statistical analysis techniques have been pioneered and successfully applied in areas such as fisheries oceanography and ecology. Finally, and perhaps most importantly, an interdisciplinary approach to studies of population variability is becoming increasingly commonplace, bringing together the specialized disciplines of fisheries biology, oceanography, meteorology, and statistics.

The very strong 1982–1983 El Niño – Southern Oscillation (ENSO) event galvanized scientific investigation into the dynamics and impacts of ENSO. The study and understanding of decadal-scale climate variability is still in its infancy. In many ways, it resembles the early investigations into ENSO. At this stage, most studies are retrospective characterizations of physical variability and coincident ecosystem response. As the relationship between ENSO and the Pacific Decadal Oscillation becomes better understood, modeling efforts may ultimately allow us to predict decadal-scale regime shifts.

In summary, while we believe that we have developed an assessment method and a management strategy that are robust to environmentally driven changes in the Pacific halibut stock, understanding the reasons for these changes is nonetheless of great scientific interest. Research to this end is being conducted under the auspices of several national and international programs, including Global Ocean Ecosystem Dynamics (GLOBEC), the North Pacific Anadromous Fisheries Commission (NPAFC), and the North Pacific Marine Science Organization (PICES). In this manner, a comparative research approach is being implemented, with studies across species, regions, and time periods providing the "replicate" observations needed to test hypotheses.

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An empirical model of fleet dynamics in New England trawl fisheries

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Abstract: Regulations and changes in market and environmental conditions that change the profitability of one fishery or area will result in a redistribution of fishing effort among alternative fisheries or areas. The magnitude of this effort displacement will depend on the relative profitability of the alternatives for the individual fishers affected. When fishing areas and fishers are heterogeneous, simple aggregate effort models such as those based on ideal free distribution theory may provide inaccurate predictions. We present an empirical model of individual vessel fishery and location choice based on trip data for a group of over 400 large trawlers fishing in New England. The model uses lagged average revenue rates for different alternatives and the individual vessel's past behavior to predict choice of species group and fishing location on a trip-by-trip basis. This model is used to predict aggregate effort levels in different fisheries and areas over time.

Résumé : La réglementation et les changements des conditions économiques et environnementales qui modifient la rentabilité d'une pêche ou d'une zone peuvent occasionner une redistribution de l'effort entre d'autres pêches ou zones. L'ampleur de ce déplacement de l'effort dépendra de la rentabilité relative des options pour chacun des pêcheurs touchés. Quand les zones de pêche et les pêcheurs sont hétérogènes, les modèles simples d'agrégation de l'effort, comme ceux qui se fondent sur une théorie de la libre distribution idéale, peuvent donner des prédictions fausses. Nous présentons un modèle empirique du choix de la pêche et de la zone, par bateau, à partir de données sur les campagnes de pêche d'un groupe de plus de 400 gros chalutiers de Nouvelle-Angleterre. Le modèle utilise les taux de revenu moyen retardé de diverses options et le comportement passé de chacun des bateaux pour prédire le choix des groupes d'espèces et du lieu de pêche campagne par campagne. Ce modèle sert à prédire les niveaux globaux d'effort dans les différentes pêches et zones sur une certaine période.

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Introduction

Understanding the response of fishers to changes in biological, economic, and regulatory conditions in fisheries is critical to designing management plans that will both protect the resources and provide economic benefits to fishers and consumers. This is particularly true in fisheries managed without direct controls on total effort or catch. Failure to anticipate and respond to fishers increasing or decreasing and redistributing their effort among fisheries and areas may reduce economic benefits and can result in severe declines or collapse of fish stocks. Fisheries management plans are generally applied to individual stocks of particular species or jointly targeted groups of species. For the most part, fisheries are managed independently, although they may be prosecuted in areas or by groups of fishers that overlap. Changes in biological and economic conditions or regulations that reduce or increase the relative profitability of such fisheries will result in a redistribution of fishing effort. Area closures, which are becoming an increasingly popular management tool, will almost certainly change the distribution of fishing effort. In such cases, we cannot predict effort levels in one fishery without considering conditions in alternative fisheries from which or to which fishers may reallocate their effort.

Economic theory predicts that the distribution of fishing effort will be determined by the expected economic returns to individual fishers from fishing in alternative fisheries and locations (Gordon 1954). In a single-species fishery where fishers and fishing areas are homogeneous, we could reasonably expect effort to be distributed in such a way as to equalize average profit rates among areas. This has been demonstrated empirically in some fisheries (e.g., see Hilborn and Ledbetter 1979; Campbell et al. 1993; Gillis et al. 1993). In a multispecies fishery with costs and catch expectations that differ among fisheries, areas, and individual fishers, predicting the distribution of fishing effort becomes much more complex. The relative attractiveness of fishing in a particular fishery and location may still be determined by expected profit rates, but profit expectations for the same area may be quite diverse across fishers. In this case, observed profit rates may vary considerably between fisheries and areas.

The purpose of this research is to develop a dynamic model that can be used to predict how changes in biological,

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Table 1. Total revenues and percent share of total revenue for large trawlers by large ports and regions for all recorded landings from New England fisheries from 1990 to 1993.

Ports and port groupings	Revenues, 1990–1993 (millions of dollars)	% of total revenues
Northeast Maine	6.36	1.2
Portland Maine	62.69	12.1
Gloucester and Newbury Port, Mass.	79.49	15.3
Boston, Mass.	45.84	8.8
Plymouth and Scituate, Mass.	0.67	0.1
Cape Cod and Islands, Mass.	9.50	1.8
New Bedford and Bristol County, Mass.	215.48	41.6
Newport, R.I.	18.83	3.6
Point Judith, R.I.	76.80	14.8
New London, Conn.	0.06	0.0
Long Island, N.Y.	0.47	0.1
New Jersey	2.11	0.4
Virginia	0.26	0.1
Total	518.57	100.0

economic, and regulatory conditions will impact the level and distribution of fishing effort in the New England trawl fisheries. Heterogeneity among individual fishers or vessels suggests that prediction of aggregate effort levels should begin with predictions at the individual level. In this paper, we present a model of individual fishery and location choice for a fleet of 484 large trawlers in a multispecies, multiarea fishery in New England. This model of fleet dynamics is used to explain the behavior of individual fishers and to predict the distribution of aggregate fishing effort among alternative fisheries and areas over time.

Background on the New England trawl fisheries

The Northeast multispecies fishery underwent a severe decline in the early 1990's. In response the New England Fishery Management Council (NEFMC) introduced a number of measures intended to reduce fishing mortality on the depleted stocks and promote their recovery. These included large area closures on Georges Bank and in Southern New England, a moratorium on entry into the fishery, individual vessel effort limitations (days at sea), and a variety of other regulations. Direct catch quotas are not set, but additional measures will be used if necessary to ensure reduction of fishing mortality to target levels (NEFMC 1996).

The primary purpose of the new measures is to rebuild the spawning stocks of the principal groundfish stocks (Georges Bank cod, haddock, and yellowtail flounder, Gulf of Maine cod, and Southern New England yellowtail flounder); however, the management plan also governs a number of other stocks. These include stocks of pollock, winter flounder, witch flounder, windowpane flounder, American plaice, red, white, and silver hake, ocean pout, and redfish. These stocks are jointly fished by a variety of gears including gill nets and longlines, but otter trawlers account for the great majority of catch. Many of the trawlers also participate in a number of other fisheries including squid, skate, summer flounder, shrimp, swordfish, scallops, monkfish, and, more recently, mackerel and dogfish. The near collapse of the important groundfish stocks and subsequent regulatory changes are leading to changes in the distribution of effort between species groups and areas. Effort has been displaced from primary groundfish species on Georges Bank to other areas in the Gulf of Maine and Southern New England. Effort redistribution will likely continue as markets are developed for "underutilized" species and as stock levels rise and fall in alternative fisheries. The current trend may reverse at some point when groundfish stocks on Georges Bank recover. A more complete understanding of the fleet dynamics in these fisheries will give fisheries managers the ability to better predict and adjust for changing effort levels among fisheries to avoid future collapse and increase their value.

We focus on a group of 484 large trawlers that accounted for about 65–70% of the documented landings from the multispecies fishery during the 1990–1993 period. This fleet includes all large trawlers with documented landings from New England fisheries in the 1990–1993 period. These vessels range from 50 to over 200 gross tons (1 short ton = 0.907 metric ton) and land fish from northeast Maine down to Long Island with occasional landings as far south as Virginia. The majority of the fleet's revenues come from landings in Portland, Maine, Gloucester, Boston, and New Bedford, Massachusetts, and Point Judith, Rhode Island, but other ports account for a significant portion of revenues (Table 1). The revenues of the fleet as a whole come from a wide variety of species (Table 1). Some vessels tend to specialize by species or species groups, but others move between the fisheries throughout the year depending on the relative abundance, prices, and regulations. All have access to several fisheries in the area and in some cases may land catch from multiple fisheries in a single trip.

We model the fleet's movement between different areas of the multispecies fishery and fisheries for red and silver hake, squid, summer flounder, and skate. Although red and silver hake are part of the multispecies fishery from a regulatory standpoint, we consider them as a separate fishery because they are fished with different gear and not jointly targeted with the groundfish and flatfish stocks. The rest of the species governed by the Multispecies Fishery Management Plan are considered as one fishery in our model of fishery and location choice. No other "target" fisheries accounted for over 1% of the fleet revenues during the 1990–1993 period, the time span of our study (Table 1). Although over 5% of the fleet's revenue came from monkfish, it appears to have been an incidental catch. Monkfish was a small but significant share of revenues from a large percentage of groundfish trips but was not the primary species landed for any trips (Table 2). In contrast, skate accounted for only about 1% of actual revenues, but trips that target skate brought in 2.5% of revenues. This has likely changed now as more vessels have begun targeting underutilized species such as monkfish, mackerel, and dogfish. Scallops also accounted for a significant share of the fleet's revenue. However, movement between the scallop and groundfish fisheries is not very frequent, as it involves a major refitting of the vessel. This is

Table 2. Shares of large trawler revenues by actual species composition and by target fishery as determined by main species caught for all recorded landings from New England fisheries from 1990 to 1993.

Species group	Share of actual revenues (%)	Revenues by target fishery (%)
Mutispecies groundfish	68.7	74.7
Squid and butterfish	6.1	6.2
Fluke	2.8	1.2
Red and silver hake	3.8	3.6
Skate	1.1	2.5
Sea scallops	8.0	8.6
Monkfish	5.1	0.0
Shrimp	0.6	0.7
Lobster	0.6	0.1
Swordfish	0.5	0.5
Other	2.7	1.8

a longer term entry–exit decision rather than a trip-to-trip fishery and location choice, and consequently, we do not include scallops in our model. We use three-digit statistical areas (Fig. 1) to partition the fisheries into discrete spatial areas for the purpose of designating location choice.

Methodology

There is a limited literature on modeling individual fishery and location choice and entry–exit decisions in commercial fisheries (e.g., see Bockstael and Opaluch 1983; Eales and Wilen 1986; Dupont 1993; Ward and Sutinen 1994) on which we will build. We extend the modeling approach applied in this literature to accommodate fishery and location choice on a trip-by-trip basis in a multispecies, multiarea fishery. The resultant individual-level behavioral models are used to predict aggregate temporal and spatial fishing effort distribution for the overall trawler fleet. Effort distribution predictions can be updated as the characteristics and size of the fleet, as well as the biological and regulatory status of the fishery, change.

We use discrete choice random utility models to model choices. Individuals are assumed to choose among several discrete alternatives (e.g., fisheries and (or) areas). We observe which alternatives they choose and assume that the alternatives chosen are those that generate the highest expected utility for them. For a given individual, the probability that a particular alternative k is chosen can be expressed as

$$(1) \qquad P(k) = P\left[U_k \geq \max_{k' \in C} U_{k'},\ \forall k \in C,\ k \neq k'\right]$$

where U represents an indirect utility function for choice k for a specific individual. The utility function is typically specified as a linear function of observable variables that are assumed to impact the relative utility of alternative choices. Parameters of the utility function that maximize the likelihood of the observed choices can be estimated using various discrete choice statistical methods. Once the parameters of the utility function are estimated, it can be used to predict the relative probabilities of individuals choices among the alternatives open to them.

We constructed our models of expected utility based on the literature and on ethnographic interviews with skippers of large trawlers from Rhode Island, Massachusetts, and Maine. Ethnographic interviewing refers to an interviewing technique developed to allow researchers to describe other cultures while minimizing the bias introduced by the researcher's preconceived notions and unfamiliar wording of questions (Spradley 1979). The model includes, but is not limited to, variables that are assumed to impact expected profit. We describe the variables included in the model and the rationale for including them below before proceeding to a more formal discussion of how the model is estimated statistically.

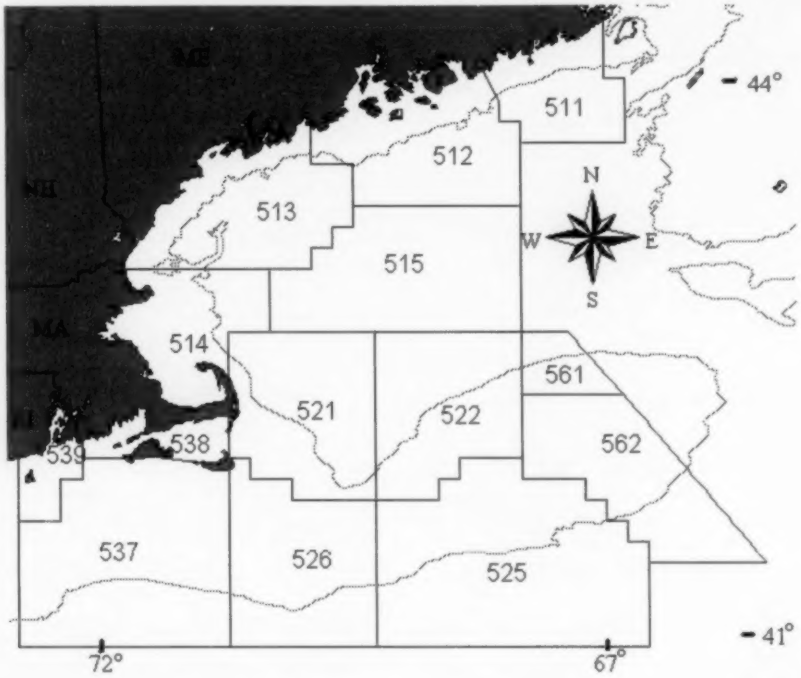
Fishers, whether by their own experience or by communication with other fishers, are assumed to possess information about recent average revenue rates in different areas and fisheries. Our interviews revealed that skippers network in groups of varying sizes and the groups themselves may change rapidly. Wilson (1990) showed that the optimum "club" size for sharing information would depend on the abundance and patch size of fish aggregations. Allen and McGlade (1986) suggested that fishers may be categorized into leaders and followers and that the degree to which the second group will follow leaders depends on the level of information flow.

Since we cannot explicitly model the information flow among individual fishers, we use as a proxy the average revenues for all vessels from the large trawler fleet that were fishing a given fishery/area during the relevant period. This information enters the predictive model as an average revenue per day for trips landing fish from that fishery/area over the prior 10-day period. Skippers indicate that the fish follow similar migratory patterns from one year to the next, so we also include 1-year lags of average revenue per day for the particular fishery/area as an explanatory variable. The year-lagged average revenue is based on trips that landed or departed within a 20-day period surrounding the departure date the prior year. The cutoff of 10 days prior to departure was chosen because skippers indicated that information about the prior location of aggregations of fish would have little value after that length of time.

We use average revenue rates rather than catch rates because of the differing values by species and size of fish. The fleet of large trawlers from which average revenue rates are calculated is highly diverse, with vessels ranging from 20 to over 300 gross tons, from under 20 to over 60 m in length, with engines ranging from 200 to over 2000 hp (1 hp = 745.7 W). The average revenue rates used as explanatory variables are based on the revenues per standardized day on the fishing grounds net of steam time to and from port. We utilize a translog production function of tonnage, length, horsepower, and age to standardize fishing effort. See the Appendix for a more detailed account of how effort is standardized.

In many cases, there were no recorded trips to an area over the period for which average revenue must be calculated. There are three general approaches to handle this problem (Little 1992). The first is to throw out all observations for which there are missing data. This would be too

Fig. 1. Primary fishing areas for New England trawl fisheries: Gulf of Maine (areas 511–515), Georges Bank (areas 521, 522, 525, 526, 561, and 562), and Southern New England (areas 537–539).



costly, however, as there is no recorded effort in at least one of the 41 fishery/area alternatives at almost any time of year. Another alternative is to substitute an imputed value. A third alternative is to substitute an arbitrary value (commonly zero) in place of the missing value and include an additional dummy variable that takes a value of 1 in the case of missing data and zero otherwise (see Cohen and Cohen 1975 for a discussion of this method). We could not find a satisfactory method of imputing the average revenue variable and chose the third method using zero as a placeholder for the missing variable and including a dummy variable to indicate missing data.

Despite the inherent riskiness of their chosen profession, some studies have indicated that fishers are risk averse. If so, we might expect to find that, all else equal, fishers would prefer fishing alternatives in which the variability of returns is lower. We test this hypothesis by including the coefficient of variation of standardized average revenues as an explanatory variable. A negative parameter on this variable would indicate risk aversion and a positive sign would indicate risk-seeking behavior.

We include steam time from the port of landing to the fishing area divided by the expected duration of the trip as a variable to account for the implicit opportunity cost of time spent steaming rather than fishing on a given trip. Expected trip duration is based on the average trip length by fishery, area, and month. Steam time is divided by trip duration because it is the proportion of the trip spent in transit rather than the total steam time that is relevant to assessing the expected profitability of the trip (e.g., 24 h of transit for a 10-day trip may be acceptable, but it would not be for a 3-day trip).

We include lagged total effort for the fishery/area alternative as an explanatory variable. Total effort may indicate the overall size of the fish stock in the area whereas revenue per day indicates the stock's density and species composition. Although it is somewhat ambiguous how this variable should enter the expected utility function, we hypothesize that marginal catch rates fall less quickly the larger the overall stock of fish in an area so that information about past catch rates will have value longer. A larger number of vessels fishing an area also increases the chances that any individual would acquire information about the presence of fish there and increases the quality of the information (i.e., high revenue per day for one vessel may be a fluke, but this is unlikely if 30 vessels report good catches). Vignaux (1996) found that vessels in the New Zealand hoki fishery tended to move toward areas where other vessels had congregated the prior day. We also include 1-year lags of total effort, since fish tend to follow similar annual migratory patterns from one year to the next.

The surface area for a given fishery/area alternative is also included as a variable. Like total effort, this variable is a partial indicator of the size of the total fish stock in the area. In addition, a larger area will reduce crowding externalities. During certain periods, spawning closures prohibited fishing in some areas. In no case was an entire statistical area closed, but large parts of some were. We include in the model a dummy variable to indicate when part of an area was closed due to a spawning closure.

Our interviews of skippers suggest that they tend to follow their own historical patterns of fishery and location choice. They have developed time- and location-specific information that increases their expected revenues by decreasing the time spent searching for aggregations of fish. A fisher's expectation of revenues might be higher or lower than average revenues for a given time and location depending on his or her experience in the area. Including an individual's historical fishery and location choice is intended to capture this implicit advantage or disadvantage resulting from experience with a given fishing alternative or lack of it. As Bockstael and Opaluch (1983) hypothesized, the tendency of fishers to follow their own historical patterns may also be due nonmonetary factors such as family tradition and simple inertia. Our current modeling framework does not allow us to differentiate these behavioral motives, however. This information enters the model in dummy variables that take a value of 1 for alternatives that were chosen on prior trips. Six of these "habit" variables were constructed: indicators of whether the individual had chosen the specific fishery/zone/area alternative, the zone/fishery combination, or the same fishery on a trip that landed within the last 10 days of the start date of the observed trip or within a 20-day period surrounding the start date the prior year.

Like Eales and Wilen (1986), we utilize a nested model structure assuming a hierarchy of decisions; first, a fishery and zone choice (e.g., groundfish on Georges Bank or squid in Southern New England) and then an area choice within the larger zone. Areas are the three-digit statistical areas (Fig. 1) that range in size from less than 2700 to over 25 000 km^2. These areas are irregular in shape and size, but conform reasonably well to distinct areas of the fishery as described by skippers in our interviews with them. For this reason, we consider them superior to a spatial division of the fishery using a regular grid. We were unable to examine location choice on a finer scale due to limitations of the data. Although the data list a location on a quarter-degree square grid, only one such location is given per record. Many trips are several days long and it is not realistic to assume that the entire trip was spent in one quarter-degree area. It is not uncommon for a vessel to fish more than one statistical area on a trip, but port agents would then separate the trip into multiple records. More recent data have been collected using a logbook system. It is possible that a model with a finer spatial resolution could be estimated with these data. However, estimating such a model would be problematic because of the large size of the choice set. Fishers are assumed to make a decision between one of 41 discrete fishery/area alternatives prior to leaving port.

Each fishing alternative has an expected utility based on a vector of explanatory variables and an error term. Assuming that the utility function from eq. 1 can be divided into a systematic component, V, and a random component, ε, the discrete choice probabilities can be written

$$(2)\qquad P(k) = P[V_k(\cdot) + \varepsilon(\cdot) \geq V_{k'}(\cdot) + \varepsilon(\cdot),\ \forall k \in C_i,\ k \neq k'].$$

Estimation of eq. 2 from observed choices leads to predicted choice probabilities at the individual and aggregate level (Manski and Lerman 1977). Some behavioral assumptions are required to estimate eq. 2. Typically, choices are assumed to be independent and mutually exclusive such that the ratio of the probabilities of choosing any one alternative over another is not affected by the attributes of other alternatives in the choice set. The random components of the indirect utility functions, ε, are assumed to be independently and identically distributed (iid) with the Gumbel (type I extreme value) distribution (see Ben-Akiva and Lerman (1985, p. 104) for a description of the properties of this distribution and a formal derivation of the multinomial logit model). Given these assumptions the parameters of the utility function can be estimated with the multinomial logit model (Luce 1977). Combining the arguments of the utility function into a vector $\mathbf{x_k}$ with parameter vector beta, β, the multinomial logit choice probabilities can be expressed as

$$(3)\qquad P(k) = \frac{\exp V_k(\cdot)}{\sum\limits_{k \in C} \exp V_k(\cdot)} = \frac{\exp \beta' x_k}{\sum\limits_{k \in C} \exp \beta' x_k}$$

A critical and limiting assumption of the multinomial logit model is that the relative probabilities of choosing two alternatives are independent of any other alternatives that may exist. This assumption is violated if some subsets of alternatives share unobserved characteristics. In this case, it is assumed that there may be unobserved characteristics common to areas within a given zone. We therefore use a generalization of the multinomial logit model known as the nested logit model to estimate our fishery/location choice model. The nested logit model accommodates cases where the random component for groups of alternatives may be correlated.

We specify a two-level model where individuals first choose a fishery/zone combination, j, at the highest level of the model and an area, k, within the larger zone at the lowest level. The utility function may now contain variables in a vector, $\mathbf{z}$, that differ across fishery and zones, but not across alternatives within the zone, and a vector, $\mathbf{x}$, of variables that differ across alternatives within a particular fishery and zone. It is important to note the level at which the different variables used enter the model. The variables for "same fishery/zone" and "same fishery" enter into the model at the upper level in the $\mathbf{z}$ vector of variables from eq. 5. The "same area" variables, steam time, and all of the choice-specific variables for which parameter estimates are shown in the second block of rows enter into the model at the lower level, which is conditional on fishery and zone choice. This is the $\mathbf{x}$ vector of variables in eq. 4.

The choice probability of the multinomial logit model is now redefined as the conditional probability of area k in fishery/zone j, $k|j$ (Greene 1995):

$$(4)\qquad P(k|j) = \frac{\exp \beta' x_{k|j}}{\sum\limits_{k \in C_j} \exp \beta' x_{k|j}} = \frac{\exp \beta' x_{k|j}}{\exp J_j}$$

where $\mathbf{x_k}$ is the vector of variables that characterize alternative k, β is the parameter vector to be estimated, and

$$J_j = \log \sum_{k \in C_j} \exp \beta' x_{k|j}$$

Table 3. Parameters and summary statistics for a fishery and location choice model using a 25% random sample of all large trawlers trips in New England areas from 1990 to 1993.

		SE	$z = b/\mathrm{SE}$
Individual and choice-specific variable coefficients			
Same fishery/area past 10 days	1.75	0.03	54.78
Same fishery/area last year	1.21	0.03	37.60
Same fishery/zone past 10 days	0.80	0.06	14.32
Same fishery/zone last year	0.81	0.06	13.14
Same fishery last year	–0.52	0.06	–8.18
Steam time/expected trip length	–3.17	0.08	–42.02
Fleet average revenues per day past 10 days ($1000's)	0.10	0.01	13.11
Dummy: no trips past 10 days	–0.61	0.08	–7.86
Fleet average revenues per day last year ($1000's)	0.07	0.01	7.51
Dummy: no trips last year	–0.72	0.09	–8.02
Coefficient of variation revenues per day past 10 days	0.16	0.03	5.98
Coefficient of variation revenues per day last year	0.18	0.03	6.69
Total effort past 10 days (100's of days)	0.29	0.03	10.73
Total effort last year (100's of days)	0.06	0.01	6.54
Surface area (ha)	0.21	0.02	10.29
Inclusive value parameters			
Southern New England groundfish	1.11	0.03	38.64
Georges Bank groundfish	1.07	0.02	49.17
Inshore Gulf of Maine groundfish	1.14	0.03	41.57
Offshore Gulf of Maine groundfish[a]	1.00		
Southern New England red and silver hake[a]	1.00		
Georges Bank red and silver hake	2.29	0.09	24.22
Inshore Gulf of Maine red and silver hake	1.50	0.07	22.85
Southern New England fluke	1.14	0.06	20.67
Georges Bank fluke	0.99	0.09	10.81
Southern New England skate	1.08	0.13	8.31
Georges Bank Skate	0.65	0.05	13.70
Inshore Gulf of Maine skate[a]	1.00		
Southern New England squid	1.17	0.03	34.45
Georges Bank squid	0.66	0.07	9.35
Log likelihood function	–19 612		
Restricted log likelihood	–39 460		
Pseudo-R^2	0.50		

[a] These inclusive value parameters are fixed at a value of 1.0 because there is a single area choice in that branch.

and is known as the inclusive value for fishery/zone *J* and represents the composite utility of the choices within branch *j*.

The probability of choosing a particular fishery/zone *j* is

$$(5)\qquad P(j) = \frac{\exp(\gamma' z_j + \sigma_j J_j)}{\sum_{j \in C} \exp(\gamma' z_j \sigma_j J_j)}$$

where σ is the inclusive value parameter, $\mathbf{z}_j$ is a vector of variables that differ across fishery and zone, but not across area alternatives, and $\boldsymbol{\gamma}_i$ is the estimated parameter vector or the variable vector $\mathbf{z}_j$. The unconditional probability of choosing a given alternative is the product of the probability of choosing the fishery/zone, and the conditional probability of choosing the area given the zone and fishery choice, calculated as $P(j) \cdot P(k|j)$ from eqs. 4 and 5. Commercially available software is used to estimate the parameters of this model simultaneously using full information maximum likelihood methods (we use LIMDEP™, a product of Econometric Software, Inc.).

Results

We estimate a nested logit model with 41 different fishery/area alternatives (Table 3). Our analysis is based on 39 292 trips made by large trawlers in the years 1990–1993. Because of the large size of the data set, we split it into four random samples of approximately equal size. This allows us to test the robustness of the model across data sets. The same model was run with the other three randomly sampled quarters of the data and provided parameter estimates that are not significantly different across samples at a 5% significance level. With a pseudo-R^2 of 0.50, the explanatory power of the model is quite high for a discrete choice model (Table 3). The pseudo-R^2 is commonly used to assess the predictive power of discrete choice models. It is calculated as 1 minus the ratio of the unrestricted and the restricted (with zero parameters) log likelihood functions. All variable parameters are significant at the 99% confidence level (Table 3). The dummy variable for area closures and the habit variable for same fishery past 10 days were not significant and are not included in the final model.

Many of the variables in the model are correlated to some degree. Recent and year-lagged total effort in particular are highly correlated. This can bias the individual parameter estimates, but the choice probability predictions remain unbiased. We ran a variety of restricted models leaving out different explanatory variables to see if a smaller set would still provide the same predictive power and eliminate potential problems of multicollinearity. However, eliminating any variables other than the area closure variable and the "same fishery past 10 days" variable reduces the predictive power of the model significantly as determined by a likelihood ratio test.

We also ran models without the habit variables to explore their role in generating the model's predictive power. The pseudo-R^2 for a model without the habit variables fell to 0.35, indicating a major decline in the model's predictive power.

Prediction of aggregate effort

The model's predictive power can be evaluated in a number of ways. The model can be used to compute a set of choice probabilities for each of the 41 alternatives for each observation. We use the parameters estimated with the first random quarter of data (Table 3) to calculate the predicted probability for each of the 41 alternative fishery/location choices for the observations in the second quarter of data. The observed choice agrees with the choice with the highest predicted probability 43% of the time. However, prediction in this manner fails to make use of much of the information that the model provides.

Our principal purpose is to predict aggregate effort for the fishery/areas. Therefore, a more useful evaluation of the model is to examine the correlation between observed and predicted aggregate effort. Again, we use parameters estimated with the first quarter of data to make predictions of choices for observations in the second quarter. We sum the probabilities of each fishery/area alternative multiplied by expected trip length over all observations that fall within a discrete period. For example, if during a discrete period of time, we had five vessels fishing, and we predict that each had a 20% probability of fishing each of five locations, we would predict that one vessel would go to each location. If the expected trip length were 5 days for all choices, we would predict 5 days of effort for each alternative. The predicted aggregate effort is then compared with the observed aggregate effort levels for that fishery/area during that period. We can evaluate the ability of the model to predict the trend of aggregate effort over time by measuring the correlation between predicted and aggregate effort. Over the range of fishery/area alternatives, the simple correlation between observed and predicted effort levels ranges from negative to as high as 97%. The correlation is generally high for fishery/area alternatives that absorb a significant proportion of total effort. A weighted average of correlation, weighted on the basis of observed total effort levels, indicates an average correlation of 79% between observed and predicted effort levels.

A more intuitive feel of the model's predictive power is demonstrated by comparing the predicted and observed levels of total fishing effort at different levels of spatial aggregation. We compare observed and predicted aggregate monthly effort in standard vessel days for large trawler groundfish trips for area 521, the most heavily fished area on Georges Bank (Fig. 2*a*), for groundfish large trawler effort for all of Georges Bank, which includes six large statistical areas (Fig. 2*b*), and for all large trawler groundfish trips in the Northeast (Fig. 2*c*). As would be expected, predictions at higher levels of aggregation are more accurate. Groundfish trips account for the great majority of fishing effort, but whiting and squid are also quite large fisheries for trawlers. Observed and predicted aggregate monthly large trawler efforts for whiting (Fig. 3*a*) trend quite closely, but predictions of the trend of total effort is relatively poor for the squid fishery (Fig. 3*b*).

Discussion

The estimated parameters of the model (Table 3) generally conform to theoretical predictions and the behavioral model elicited from interviews with skippers. With the exception of the variable on fishery choice past 10 days, the habit variables that indicate whether the individual had chosen the fishery, zone, or area in the past are all significant and are by far the most influential variables in the model. With the exception of the variable on fishery choice the prior year, the parameters all have positive signs, suggesting a high degree of fidelity to past fishing patterns. The negative sign on fishery choice the prior year indicates that if they choose to fish in a different zone than they did the prior year, fishers are likely to also switch species groups. As we mention in the methodology, these variables may be capturing simple inertia as well as an individual's tendency to return to areas where he or she has experience and thus potentially a comparative advantage. This dynamic formulation can result in the confounding of structural state dependence with habit persistence and spurious state dependence (Heckman 1981). While specifications have been developed to address these problems (e.g., see Hensher and Le Plastrier 1985; Rishin et al. 1996), they are quite complex and appear impractical for our model due to the large size of our data set, the large number of choice alternatives, and the nested structure of the model.

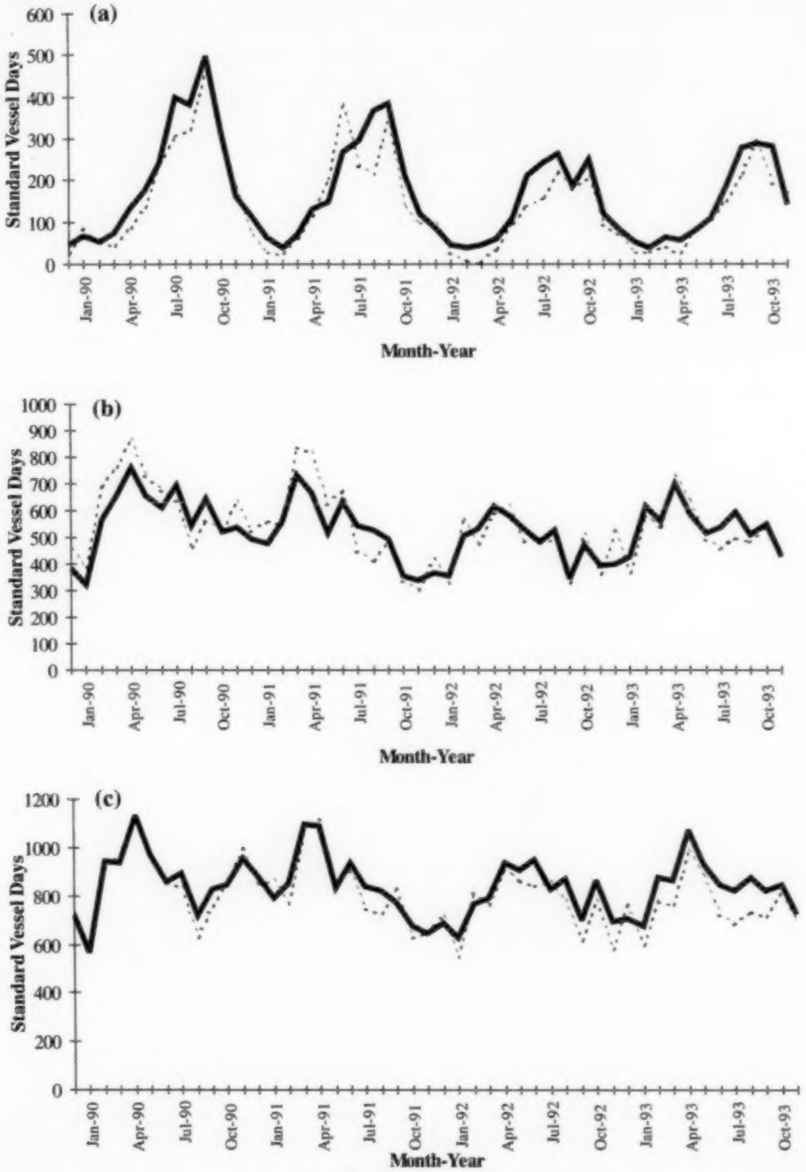
Nevertheless, most fishers do move between areas and fisheries frequently as relative conditions change. The parameters on average revenue per day for trips landing the past 10 days and the same period the prior year are also significant and positive. This indicates that fishers move toward areas with higher revenue; however, the magnitude of the parameters is quite small in comparison with those on the habit variables. A comparison of the parameters for the average revenue and habit variables for recent trips suggests that a very large increase in average revenue would be required to induce an individual to change locations from that of the prior trip. There are a number of possible explanations for this. We are not able to explicitly model information flow between vessels, so, while a skipper might respond strongly to lagged revenue information he or she receives, the skipper may be unaware of the average revenue of the entire fleet fishing in an area, particularly those where he or she does not typically fish. In many cases the average revenue variable is based on only one or a few trips. In such cases the likelihood of an individual being aware of it is correspondingly smaller. The likelihood of the average revenue being an accurate indicator of conditions over the entire area may also be small when it is based on only one or a few trips.

Our results indicate that fishers do not seek to reduce risk by choosing areas where revenue rates are less variable. The parameters on the coefficients of variation of average revenues are positive, which suggests risk-seeking behavior. This may indicate that fishers are responding to news of a few highly successful trips rather than considering average revenue rates. Fishers may attempt to reduce risks in other ways. In our interviews with skippers, several mentioned that they attempt to reduce risk due to variable prices by targeting an assortment of species, thereby creating a catch portfolio less susceptible to large swings in price of individual species.

The parameters on lagged total effort in a fishery/area are also positive. As we discussed above, there are two possible reasons to expect this. Higher effort levels may increase the chance that any individual would have acquired information about conditions in the area. Second, higher effort levels may also be a proxy for the total quantity of fish in the area which would result in a smaller marginal decline in catch rates as more effort is applied to the area. This would reduce the risk that an area would be fished out when an individual arrives there. We are unable to differentiate these effects, however.

The parameters on the variables that indicate that no trips were recorded for that fishery/area in the past 10 days or the

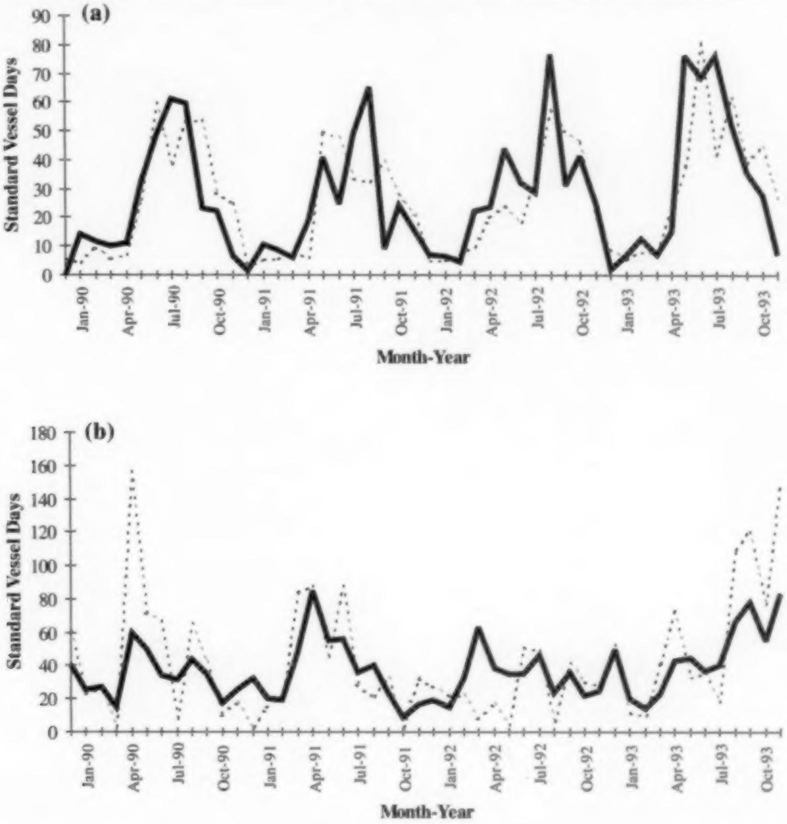
Fig. 2. Observed (broken line) and predicted (solid line) groundfish effort in standard vessel days at different levels of spatial aggregation: (*a*) statistical area 521 off Cape Cod only, (*b*) all of Georges Bank, and (*c*) all New England statistical areas. Model parameters are estimated using a 25% random sample of all trip observations, and predictions are then made out-of-sample using the predetermined parameter estimates on a second 25% random sample of the data.



surrounding period the prior year are significant and have negative signs. These variables are proxies for missing average revenue variables which cannot be calculated in the absence of recorded trips. The negative sign of the parameters suggests that they are acting as more than a proxy for the missing average revenue variable (which cannot be less than the substituted value of zero). The probable explanation for this is that at certain times of year in some fisheries and areas the target species is simply not present in significant aggregations and, consequently, fishers will almost never go to those areas. This is particularly true for squid, whiting, and fluke in specific areas.

A dummy variable indicating that a portion of the fishing area was closed (these were seasonal spawning area closures) had a negative parameter but was not statistically significant and was not included in the final model. However, the parameter on the surface area of the fishing area is significant and positive, suggesting that, all else equal, more ef-

Fig. 3. Observed (broken line) and predicted (solid line) effort in standard vessel days for (*a*) red and silver hake (referred to as whiting) and (*b*) squid. Model parameters are estimated using a 25% random sample of all trip observations, and predictions are then made out-of-sample using the predetermined parameter estimates on a second 25% random sample of the data.



fort is attracted to larger areas. While the closures often substantially reduce the fishable area, they also tend to attract fishers who like to fish the boundary of the closure to capture fish migrating out of the closed area. These effects appear to offset each other, resulting in the lack of significance of the area closure variable.

The inclusive value parameters range from as low as 0.65 for skate on Georges Bank to as high as 2.29 for Georges Bank red and silver hake (whiting). Inclusive value parameters are a measure of the correlation of error terms and thus the similarity of unobserved attributes of alternatives within a branch of the nested logit model. As Train et al. (1989) explained, the inclusive value parameter can be interpreted as a measure of the relative substitutability of alternatives. Inclusive value parameters greater than 1 mean that, when relative conditions amongst alternative areas change, the changes in probabilities of choosing areas outside a zone or in a different fishery are greater than changes within the same fishery and zone. For example, if an area on Georges Bank is closed, some vessels will divert their effort to other areas on Georges Bank while others will move to other zones. With inclusive value parameters equal to 1 for all zones, the probabilities for all remaining location choices would increase proportionately. However, with inclusive value parameters greater than 1, the probabilities of moving to areas off Georges Bank would increase proportionately more than for alternative areas on Georges Bank. The opposite would be true if the inclusive value parameter were less than 1. The inclusive value parameters for offshore Gulf of Maine groundfish, Southern New England whiting, and inshore Gulf of Maine skate are fixed at 1 because there is only one choice in this branch of the choice tree.

For the most part, the different zones (Southern New England, Georges Bank, and Gulf of Maine) contain distinct stocks of fish. Because of the migratory patterns of the fish, at a given time of year, there are typically some areas within a fishery and zone where fishing is likely to be good and others where it is not. Given a reduction in revenue expectations in a particular area within a zone, fishers may be more likely to move to a different zone or species than to move to an area within the same zone where catch expectations are generally low at that time of year.

Conclusions

Predicting the allocation of fishing effort between species groups and areas in multispecies fisheries with heterogeneous fleets is clearly a complex matter. To assume that effort will flow between areas or fisheries so as to equalize catch or revenue rates is unlikely to provide reliable predictions even when cost differentials are accounted for. Average revenue rates in different fisheries and locations in New England vary widely at any given time and change dramatically through time. Our results showed that a skipper's experience in particular areas and fisheries impacts his or her propensity to fish there in the future. This may be due to a comparative advantage based on individual knowledge of the area, but may also reflect a tendancy to maintain traditional fishing patterns despite potential gains that might be derived from their alteration. Unfortunately, we are not able to distinguish the influence of habit versus comparative advantage with the current modeling framework, but hope to segregate these effects in future work.

Our model can be used to predict the redistribution of fishing effort as conditions and regulations in the fishery change. These predictions may be useful in adjusting policies to accommodate changes in the distribution of effort. It can also help policy makers to understand the different impacts that regulations may have on different sectors of the fleet (for equity and political reasons). The importance of recent information and fishers' own recent behavior in predicting fishery and location choice suggests that our ability to make long-term predictions may be limited. However, the model of fleet dynamics can be incorporated into a bioeconomic simulation to examine the impacts of different management policies under various environmental and market conditions over extended periods of time.

While modeling aggregate effort using an individual choice model is possible, it is complex and requires a great deal of information about conditions in the fishery and about individual fishers. Yet, simpler models based on ad hoc behavioral assumptions about aggregate effort may not provide accurate predictions of effort distributions if they fail to account for the heterogeneity of the fishers. If managers are unable to predict and respond to changes in effort levels among fisheries and areas, direct controls on total catch or effort levels may be necessary to ensure profitability and conservation of fisheries. Individual output or input controls may also be required to prevent dissipation of fishery rents through input stuffing.

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Appendix. Calculating average revenues rates for fishery/area alternatives

We assume that fishers choose fisheries and locations from among alternatives at least partially based on relative rates of revenue per unit effort. However, the landings data from which this variable is constructed are generated by vessels with diverse characteristics landing fish in several different ports. To create a variable relevant to different vessels from different ports, it is necessary to calculate average revenue per standardized unit of effort net of steam time.

To estimate steam time, we use ArcInfo (a geographic information systems software package made by Environmental Systems Research Institute, Inc.) to calculate distances from the port of landing (smaller ports were grouped together when they were fairly close together) to specific fishing areas designated in the landings data. Based on an average steaming speed of 10 kn, the estimated round-trip steam time was subtracted from the vessel's trip length to yield a trip length net of steam time. The resulting days on the grounds net of steam time were used in the effort standardization models and in average revenue rate calculations.

A second complication in calculating meaningful average revenue rates is to account for the different catching power of vessels. Even though we limit our analysis to large trawlers over 18 m in length and 50 gross tons, there is great diversity in length, tonnage, and horsepower, and it is clear that this diversity greatly affects the relative catching power of vessels. A large vessel's 1000 kg of cod per day not have the same implication about fish abundance as a smaller vessel with the same catch rate. Models used to standardize effort generally use catch rates as the dependent variable (e.g., see Gavaris 1980). However, the species and size makeup of the catch also affects the catch rate. Skippers indicate that they have some ability to target specific species within the groundfish complex, although it is difficult to observe this in the landings data, since trip landings are the result of multiple tows, and skippers will often attempt to offset price risks by targeting a diversity of species on a single trip. Preliminary analysis of groundfish trips shows a significant inverse relationship between price per pound and catch (in weight) per day. This indicates that fishers may trade off quantity for quality in choosing where to fish. Many skippers maintain that they really fish for dollars, not fish, and it seems that revenue rates rather than catch rates are the variable of true interest in the location choice decision.

If our aim in standardizing effort is to create an average revenue variable that will have the same meaning for vessels with different characteristics, it is clearly necessary to standardize effort in terms of the relative ability of different vessels to generate revenues. We are unaware of any literature on standardization of vessels on the basis of their ability to generate revenues. We modify the standard framework for effort standardization by simply replacing catch with revenue as the dependent variable. Given the standard general production function (assuming dollars rather than fish are being produced), we have

$$Y = pqEA \text{ or } Y/E = pqA$$

where Y is revenues, p is price, E is nominal effort, A is stock level, and q is a catchability coefficient that is some function of vessel characteristics. A flexible form for this production function is the translog function (Kirkley et al. 1995), which, after taking logs, yields the following model for estimation given four vessel characteristics: horsepower (HP), tonnage (TONS), length (LEN), and age (AGE):

$$\begin{aligned}\log(Y/E) = {} & b_0 \ln(pA) + b_1 \ln(\mathrm{HP}) + b_2 \ln(\mathrm{TONS}) \\ & + b_3 \ln(\mathrm{LEN}) + b_4 \ln(\mathrm{AGE}) \\ & + b_5 \ln(\mathrm{HP})^2 + b_6 \ln(\mathrm{TONS})^2 \\ & + b_7 \ln(\mathrm{LEN})^2 + b_8 \ln(\mathrm{AGE})^2 \\ & + b_9 \ln(\mathrm{HP})\ln(\mathrm{TONS}) + b_{10} \ln(\mathrm{HP})\ln(\mathrm{LEN}) \\ & + b_{11} \ln(\mathrm{HP})\ln(\mathrm{AGE}) \\ & + b_{12} \ln(\mathrm{TONS})\ln(\mathrm{LEN}) \\ & + b_{13} \ln(\mathrm{TONS})\ln(\mathrm{AGE}) \\ & \qquad + b_{14} \ln(\mathrm{LEN})\ln(\mathrm{AGE}) + \varepsilon.\end{aligned}$$

We do not have observed values for p or A, but $b_0 \ln(pA)$ can be replaced with b_0D (one or more dummy variables for areas and time periods) and a constant term. This would pose a problem if our goal was to actually predict revenue rates, but if we are only concerned about vessels' relative catching power, these terms all drop out when we exponentiate and take the ratio of one vessel's catching power to another's. This relies on the assumption that the coefficients on the vessel characteristics are stable given different levels of stock abundance, different areas, etc.

The multiple R^2 for the models is 0.27 for groundfish, 0.41 for squid, 0.40 for whiting, 0.27 for skate, and 0.42 for fluke. The R^2 values indicate that the models fail to explain much of the variance in revenue rates, but we remind the reader that much of that unexplained variance is due to error in the proxy, A, for stock abundance and composition so that the multiple R^2 is not a good indicator of the accuracy of the parameters on vessel characteristics. We adjusted for heteroskedasticity by substituting a heteroskedastistic-consistent covariance matrix when estimating coefficients of the model (White 1993).

The estimated coefficients were then used to transform days on the grounds (days at sea minus estimated steam time) to standard days on the grounds for all observations used in calculating average revenue rates. The procedure we use in calculating the explanatory variable of average revenue is as follows. For each potential departure day of the 5-year period, a 10-day and 1-year lag weighted average of revenues per standard day on the ground is calculated for each fishery/area alternative. The weighting is based on the days at sea for each trip that fall within the period considered. For the 10-day lag (or trip lag), all trips that landed with 10 days prior to the departure date go into the average. Thus, a 10-day trip that started 15 days prior would get the same weight as a 5-day trip that started 5 days prior. The weighting does not give more weight to larger boats or to more productive trips, as we assume that each vessel's day on the grounds is as good an indicator of conditions as the next. For the year lag, all trips in the previous year that landed or started within the 20-day period of the departure date are included in the weighted average. Again, weighting is based on the days at sea that fall within the 20-day period.

The resulting average revenue rates are now standardized and independent of steam time. To use them in the choice model, they need to be reconverted to what they mean to the individual vessel in their choice framework. The relative marginal benefit of changes in tonnage, length, etc., for one fishery is not the same as the next. Thus, it is necessary to convert average revenue rates back to what that revenue rates would translate to for a particular vessel going into alternative fisheries. This is accomplished by multiplying the standardized revenue rate by the ratio of the vessel's catching (or revenue producing) power to that of the standard vessel. In calculating the coefficient of variation of revenues per day, this reconversion is not necessary, since it drops out of the calculation anyway.

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Seasonal dynamics of carbon dioxide and methane in two clear-water lakes and two bog lakes in northern Wisconsin, U.S.A.

Joan L. Riera, John E. Schindler, and Tim K. Kratz

Abstract: We investigated carbon dynamics in the water column and CO_2 and CH_4 exchange across the air–water interface in four closed-basin lakes in northern Wisconsin: two with clear, low dissolved organic carbon (DOC) waters and two bog lakes with stained, high-DOC waters. Clear-water lakes quickly became undersaturated following ice-out and remained undersaturated until fall turnover. Bog lakes were supersaturated in CO_2 throughout the ice-free season, although surface CO_2 concentrations dropped sharply following ice-out and increased again during autumn turnover. Differences in seasonal patterns of CO_2 were attributed to morphometry and the timing and intensity of mixing events. Ice-free season fluxes of CO_2 were 6.7 and 10.0 $mol \cdot m^{-2}$ in the bog lakes and 1.2 and 0.09 $mol \cdot m^{-2}$ in the clear-water lakes. Fluxes of CH_4 were significant only immediately after ice-out and during autumn turnover and were <0.4 $mol \cdot m^{-2}$ in the bog lakes and <0.05 $mol \cdot m^{-2}$ in the clear-water lakes. Compared with changes in carbon pools in the lakes, our results indicate rapid carbon turnover rates in bog lakes, as opposed to clear-water lakes. We suggest that allochthonous inputs of CO_2 may be responsible for this rapid turnover.

Résumé : Nous avons étudié la dynamique du carbone dans la colonne d'eau et les échanges de CO_2 et de CH_4 à l'interface air–eau dans quatre lacs à bassin fermé du nord du Wisconsin : deux aux eaux claires à faible teneur en carbone organique dissous (COD) et deux lacs de tourbière aux eaux teintées et riches en COD. Les lacs aux eaux claires se sont vite retrouvés sous-saturés après la disparition de la glace, et le sont restés jusqu'au renouvellement des eaux de l'automne. Les lacs de tourbière sont restés sursaturés pendant toute la saison libre de glace, mais les concentrations superficielles de CO_2 ont chuté brutalement après la fonte de la glace, pour remonter au moment du renouvellement des eaux de l'automne. Les différences dans les schémas saisonniers du CO_2 ont été attribuées à la morphométrie ainsi qu'à l'occurrence et à l'intensité des phénomènes de mélange. Pendant la saison libre de glace, les flux de CO_2 étaient de 6,7 et 10,0 $mol \cdot m^{-2}$ dans les lacs de tourbière et de 1,2 et 0,09 $mol \cdot m^{-2}$ dans les lacs aux eaux claires. Les flux de CH_4 étaient significatifs seulement juste après la disparition de la glace et pendant le renouvellement des eaux de l'automne et ils étaient de $<0,4$ $mol \cdot m^{-2}$ dans les lacs de tourbière et de $<0,05$ $mol \cdot m^{-2}$ dans les lacs aux eaux claires. Comparés aux changements des réserves de carbone des lacs, nos résultats indiquent que le renouvellement du carbone est rapide dans les lacs de tourbière par rapport aux lacs aux eaux claires. Nous pensons que des apports allochtones de CO_2 pourraient être responsables de ce renouvellement rapide.

[Traduit par la Rédaction]

Introduction

Recent research has shown that surface water CO_2 concentrations are highly variable among lakes and rarely in equilibrium with the overlying atmosphere (Hesslein et al. 1991; Kling et al. 1992; Cole et al. 1994; Hamilton et al. 1994; Hope et al. 1996; Kratz et al. 1997). In a comprehensive study of published and original data covering 1837 lakes with a worldwide distribution, Cole et al. (1994) found that although most samples (84%) were supersaturated in CO_2, individual lakes varied widely in CO_2 concentration. These data suggest that atmospheric exchange can be an important, but highly variable term in a lake's carbon budget. Our understanding of the sources of this variation is not well developed.

Exchanges of carbon between lake waters and the atmosphere depend essentially on the concentration of CO_2 (and CH_4) in surface waters and on wind speed. The average concentrations of CO_2 and CH_4 present in a lake at any given time are the net result of inputs (loading from terrestrial ecosystems including groundwater, in-lake production during respiration, and invasion from the atmosphere) and outputs (export to surface waters or groundwater, in-lake CO_2 consumption during photosynthesis or CH_4 oxidation, and evasion to the atmosphere). At a seasonal scale, surface concentrations and air–water exchange further depend on the succession of mixing and stratification periods. In dimictic lakes typical of north temperate regions, the flux to the atmosphere is barred by ice cover during the winter months and limited by CO_2 uptake and CH_4 oxidation in the metalimnion and epilimnion during summer stratification (Kratz et al. 1987; Hesslein et al. 1991).

In this paper, we investigated the seasonal controls of CO_2 and CH_4 concentrations and fluxes in four northern Wiscon-

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Table 1. Limnological characteristics of the study lakes.

	Crystal Bog	Trout Bog	Crystal Lake	Sparkling Lake
Area (ha)	0.54	1.09	36.65	63.87
Volume (m^3)	8962	61 600	3.8×10^6	8.8×10^6
Maximum depth (m)	2.53	7.9	20.4	20.0
Conductivity ($\mu S \cdot cm^{-1}$)	9.43	23.7	13.9	86.72
pH	4.99	4.92	6.13	7.31
Alkalinity ($\mu equiv. \cdot L^{-1}$)	17.5	54.2	25.6	655.0
DIC ($\mu mol\ C \cdot L^{-1}$)	209.2	387.5	95.8	807.5
DOC ($\mu mol\ C \cdot L^{-1}$)	774.2	1728.3	155.8	269.2
Chlorophyll ($\mu g \cdot L^{-1}$)	27.76	21.24	5.10	7.65
Trophic state	Dystrophic	Dystrophic	Oligotrophic	Mesotrophic

Note: Values are long-term means from the North Temperate Lakes Long-Term Ecological Research Project database.

sin lakes with contrasting characteristics. Two of the lakes are surrounded by peatlands and are dystrophic, having low pH and high dissolved organic carbon (DOC). In contrast, the other two lakes are oligotrophic clear-water lakes with circumneutral pH and low DOC concentrations. All four lakes are within 10 km of each other and are therefore subject to the same weather and share a similar geologic setting.

We chose to compare seasonal patterns in CO_2 and CH_4 concentrations and fluxes in dystrophic and clear-water lakes because we expected strong differences in processes controlling carbon dynamics in the two types of lakes. Dystrophic lakes tend to be smaller, shallower, and have more allochthonous DOC input than clear-water lakes. These characteristics cause differences in stability of stratification, exposure to the atmosphere, and production to respiration ratios that likely will lead to different seasonal patterns in CO_2 and CH_4 concentration and flux. Hope et al. (1996) have shown that CO_2 concentrations are well correlated with DOC concentrations in northern Wisconsin lakes. Because DOC in these lakes is correlated with wetland extent in the watershed (Hope et al. 1996), allochthonous inputs appear to be a primary determinant of CO_2 supersaturation in these lakes.

The goals of this study were to (*i*) quantify and compare the seasonal dynamics of CO_2 and CH_4 in the four study lakes and (*ii*) provide estimates of fluxes of CO_2 and CH_4 across the air–water interface. For each of these issues, we were interested in whether and how dystrophic lakes differed from clear-water lakes.

Materials and methods

Study lakes

The study lakes are primary study lakes of the North Temperate Lakes Long-Term Ecological Research Project (Magnuson and Bowser 1990) and are located in the Northern Highland Lake District in northern Wisconsin, U.S.A. (46°00′N, 89°37′W). In this region, pre-Cambrian granitic/metamorphic bedrock is overlain by about 40 m of sandy, noncalcareous till and outwash (Okwueze 1983).

All four lakes are seepage lakes where inputs and outputs occur only through groundwater or the atmosphere. Trout Bog and Crystal Bog are small, dystrophic lakes totally surrounded by peatlands dominated by *Sphagnum*, *Chamaedaphne calyculata*, *Carex oligosperma*, and *Picea mariana*. Trout Bog has a maximum depth of 7.8 m, which ensures a stable stratification throughout the summer, while Crystal Bog is shallower (maximum depth = 2.53 m) and stratifies only weakly. Both lakes have acidic waters (mean pH < 5.5) and high DOC concentrations (>700 μM). Crystal Lake and Sparkling Lake are both clear-water, dimictic lakes with circumneutral pH values. Crystal Lake has the lowest concentrations of dissolved inorganic carbon (DIC) and DOC of all the study lakes; Sparkling Lake has the highest DIC concentration (Table 1).

Carbon species concentrations

Measurements of CO_2 and CH_4 were made from one to six depths in each of the four lakes at 1- to 4-week intervals throughout the 1994 open-water season. Sampling was most intense in terms of frequency and depths sampled during the onset of stratification and just prior to fall turnover. Method details are given in Hope et al. (1996). Briefly, 40 mL of water was equilibrated with 20 mL of headspace in a syringe by vigorous shaking. CO_2 and CH_4 in the equilibrated headspace were analyzed by gas chromatography, and the concentrations of CO_2 and CH_4 in the original sample were derived from a mass balance of CO_2 and CH_4 in the syringe before and after equilibration using Henry's constant with corrections for temperature and ionic strength (Kling et al. 1992; Cole et al. 1994).

These data were augmented with values of CO_2 concentrations calculated from pH and DIC values measured during the routine monthly North Temperate Lakes Long-Term Ecological Research Project sampling program in which water from two to six depths was sampled depending on the depth of the lake. pH and DIC were measured as described in Hope et al. (1996). Care was taken to minimize gas exchange prior to measurement. Calculations of CO_2 were made using standard equations (Stumm and Morgan 1996), with constants corrected for temperature and ionic strength following Kling et al. (1991). There was good agreement between measured and calculated CO_2 concentrations in the clear-water lakes (Kratz et al. 1997), and values were pooled together for all the analyses presented here. For high CO_2 values, which occurred in bog lakes, direct measurements tended to be higher than CO_2 concentrations calculated from pH and DIC. Therefore, we estimated a regression equation for direct measures versus calculated measures (direct $[CO_2] = -19.58 + 1.34 \times$ calculated $[CO_2]$, $R^2 = 0.93$, $p < 0.001$, $n = 32$) and used it to correct values of CO_2 calculated from DIC and pH in the bog lakes only.

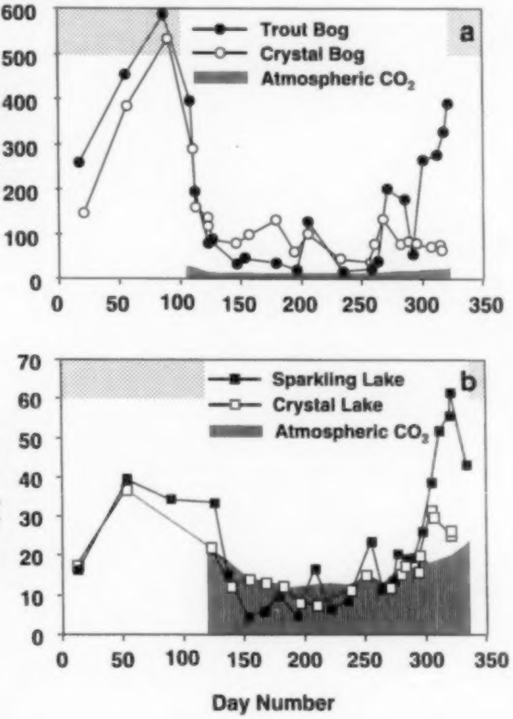
Whole-lake mass of carbon species was calculated by multiplying the concentration over a depth interval by the volume of that depth interval and then adding all the values across the depth profile. The corresponding volume-weighted concentrations and areal concentrations were obtained by dividing the total mass in the lake by the volume and the surface area of the lake, respectively.

Flux to the atmosphere

Gas flux was calculated using the stagnant film model (Liss and Slater 1974). In this approach, the mass flux of gas across the air–water interface, *F*, is defined as

$$(1) \qquad F = k \times (C_{\mathrm{sur}} - C_{\mathrm{eq}})$$

Fig. 1. Surface concentration of CO_2 in the (*a*) bog lakes and (*b*) clear-water lakes. Ice cover is shown as shaded areas at the top of each graph. The concentration of CO_2 in equilibrium with the atmosphere during the open-water period is shown by shaded areas at the bottom of each graph.



where C_{sur} is the concentration of the gas in the water at the air–water interface and C_{eq} is the concentration in equilibrium with the atmosphere; k is the gas transfer velocity (centimetres per hour), which is empirically derived using inert tracer gases, mainly sulfur hexafluoride (SF_6), in surface waters as summarized by Wanninkhof (1992). We used the empirical relationships presented by Cole and Caraco (1998). Values for kCO_2 and kCH_4 were calculated from k_{600}, which is the k measured with SF_6 and normalized to a Schmidt number (Sc) of 600, using the equation

$$(2) \qquad k_{600} = 2.07 + 0.215 \times U_{10}^{1.7}$$

where U_{10} is the wind speed at 10 m height. Values of kCO_2 were calculated from k_{600} values using the equation

$$(3) \qquad kCO_2 = k_{600} \times \left(\frac{ScCO_2}{600} \right)$$

where $ScCO_2$ is the Schmidt number for CO_2 and the coefficient a takes on a value between 1 and –0.75 depending on which process dominates diffusion; we used a value of –0.5, based on experimental determinations from Clark et al. (1995). The kCH_4 is obtained by substituting $ScCH_4$ for $ScCO_2$ in eq. 3. Schmidt numbers for CO_2 and CH_4 were calculated from empirical third-order polynomial fits to temperature determined by Jähne et al. (1987).

Wind speed was measured every 15 min at a height of 1, 2, and 3 m from 20 June 1994 through 26 October 1994 at the center of Sparkling Lake and at a height of 1 m from 29 September 1994 through 23 November 1994 at the center of Trout Bog. During the periods of common measurement, wind speed at Trout Bog was closely related to wind speed at Sparkling Lake, although wind speed at Trout Bog was consistently lower, never surpassing 3 $m \cdot s^{-1}$.

Wind speed at 10 m (U_{10}), the parameter needed in eq. 2, was estimated from available measurements. In the case of Sparkling lake, U_{10} was estimated from daily wind speed averages at 1, 2, and 3 m assuming a neutral boundary layer and a logarithmic wind profile (Arya 1988). The U_{10} at Trout Bog was estimated from measured U_1 (wind speed at 1 m height) using a linear regression ($R^2 = 0.87$, $p < 0.001$) of U_{10} versus U_1 derived from Sparkling Lake wind data and assuming that micrometeorological conditions are similar for both lakes.

For the periods when in situ measurements of wind speed were not available, U_{10} was estimated from measurements of wind speed at a nearby meteorological station (Woodruff airport) using linear regressions derived from the period of common data. The U_{10} at Crystal Lake was assumed equal to U_{10} at Sparkling lake, and U_{10} at Crystal Bog was assumed equal to U_{10} at Trout Bog. These assumptions are based on lake size and fetch and may have resulted in a slight overestimation of flux at Crystal Lake and Crystal Bog, which are more sheltered than Sparkling Lake and Trout Bog, respectively. Mean wind speed was 4.19 $m \cdot s^{-1}$ in clear-water lakes and 2.23 $m \cdot s^{-1}$ in bog lakes. In both cases, wind speed was slightly higher in autumn.

To estimate open-water season fluxes, eq. 1 was used on daily surface CO_2 and CH_4 concentrations and wind speed values. Surface concentrations between sampling dates were estimated by linear interpolation.

Results

CO_2 concentrations

Surface water CO_2 concentrations differed markedly between the bog and clear-water lakes (Fig. 1). Peak concentrations in the bog lakes were about 10-fold higher than peak concentrations in the clear-water lakes. Furthermore, surface waters of the bog lakes were at all times supersaturated in CO_2, whereas surface waters of the clear-water lakes were undersaturated or near equilibrium for most of the summer stratified period. In all of the lakes, there was a general pattern of increased CO_2 concentrations in surface water in the winter and early spring and, except for Crystal Bog, in the fall. During stratified periods, CO_2 concentrations increased with depth in all four lakes (Fig. 2). The maximum CO_2 recorded during this study, 1366 μM, or roughly 60-fold above atmospheric equilibrium, occurred in the bottom waters of well-stratified Trout Bog just prior to fall mixis. Gradual accumulation of CO_2 in the bottom waters occurred during the summer stratified season in Crystal Lake, but the other three lakes exhibited high, but variable concentrations in bottom waters during this period without evidence of a strong trend over the summer season (Fig. 2).

Changes in the total mass of CO_2 in a lake reflect the net result of all processes adding or removing CO_2. Assuming no change in lake volume, we used the volume-weighted concentration of CO_2 as a measure of total mass that could be easily compared with CO_2 concentrations along the depth gradient within each lake, as well as among lakes (Fig. 2). Immediately following ice-out, each of the lakes exhibited a net loss of CO_2. The rate of CO_2 loss in the bog lakes was about two times greater than the loss rate in the clear-water lakes during this period (Table 2). Changes in the CO_2 pools, however, reflect changes in pH besides CO_2 losses. If we look at changes in the DIC pool, loss rates were similar in all four lakes. During the summer stratified period, the

Fig. 2. Concentration of CO_2 at selected depths in (*a*) Crystal Bog,(*b*) Trout Bog, (*c*) Crystal Lake, and (*d*) Sparkling Lake. The thick line in each graph is the volume-weighted average concentration of CO_2 in the lake. Ice cover is shown as shaded areas.

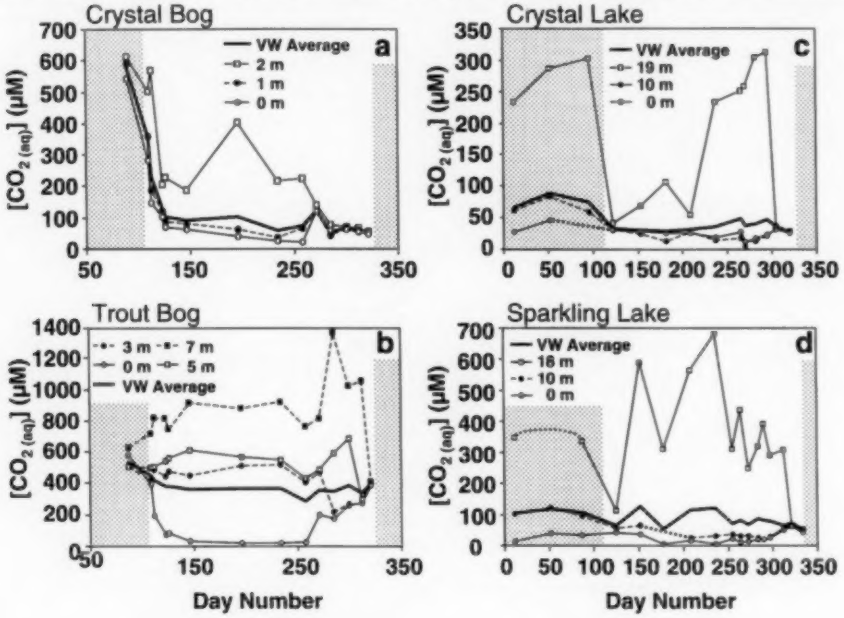


Table 2. Comparison between CO_2 exchange with the atmosphere and changes in the lakes' pools of DIC (Δmass DIC) and dissolved CO_2 (Δmass CO_2) during the spring mixing period and the autumn mixing period, the two periods of strongest exchange with the atmosphere.

Lake	Period (day numbers)	Δmass DIC ($mmol \cdot m^{-2} \cdot day^{-1}$)	Δmass CO_2 ($mmol \cdot m^{-2}$)	CO_2 flux ($mmol \cdot m^{-2} \cdot day^{-1}$)
Spring mixing				
Trout Bog	108–123	27.3	28.5	59.5
Crystal Bog	106–122	32.4	27.4	69.2
Crystal Lake	108–122	27.6	16.4	–2.0
Sparkling Lake	111–125	26.4	15.9	12.6
Autumn mixing				
Trout Bog	271–321	–0.7	–4.7	87.2
Crystal Bog	271–321	2.7	2.4	33.6
Crystal Lake	294–320	8.2	8.3	7.8
Sparkling Lake	297–320	25.4	11.0	23.0

Note: Negative values for Δmass DIC or Δmass CO_2 correspond to increases in the pools of these species. Negative fluxes correspond to CO_2 invasion.

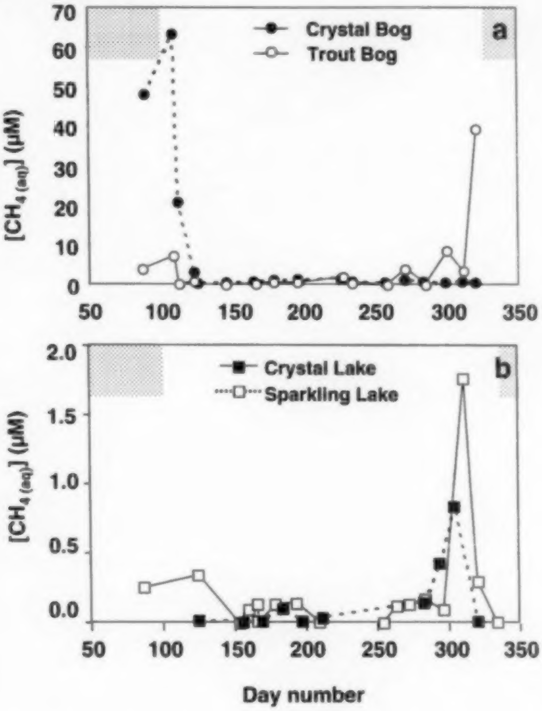
mass of CO_2 increased slightly in each of the lakes, except Crystal Bog, due to accumulation in the bottom waters. In contrast with the spring, during fall turnover the clear-water lakes exhibited greater loss of CO_2 and DIC than the bog lakes (Table 2).

CH_4 concentrations

Surface concentrations of CH_4 were low in all lakes for most of the open-water season (Fig. 3), except during mixing periods, when CH_4 stored under ice (during the winter), or in the hypolimnion (during the summer), circulated throughout the water column. The highest concentration of CH_4 in surface waters was recorded in Crystal Bog after ice-out (63.88 µM) (Fig. 3), but in this shallow bog, CH_4 concentrations remained low at all depths throughout the rest of the open-water season (Fig. 4). In Trout Bog, CH_4 concentration after ice-out was more modest (7.13 µM) (Fig. 3), but CH_4 accumulated in the hypolimnion throughout the open-water period (Fig. 4). During autumn turnover, the lake mixed completely, and CH_4 concentration reached 39.16 µM at the surface.

Clear-water lakes followed a pattern of CH_4 concentration similar to that found for Trout Bog, albeit with much lower concentrations. CH_4 concentration was above detection level (0.10 µM) after ice-out in Sparkling Lake (0.25 µM) but was undetectable in Crystal lake and remained below detection

Fig. 3. Surface concentration of CH_4 in the (*a*) bog lakes and (*b*) clear-water lakes. Ice cover is shown as shaded areas at the top of each graph.



in both lakes throughout the open-water period. During autumn overturn, CH_4 surface concentrations increased to 1.76 μM in Sparkling Lake and to 0.82 μM in Crystal Lake, only to decrease to undetectable levels before ice-on.

Depth profiles for clear-water lakes were only available for the autumn overturn period (Fig. 4). Nonetheless, data suggest that CH_4 concentrations were low throughout the water column in Crystal Lake until late September, when the concentration of CH_4 increased, reaching a maximum concentration in mid-October. Complete mixing was detected on 31 October, and by 16 November, 14 days before ice-on, the concentration of CH_4 was below 0.30 μM throughout the water column. Available depth profiles for Sparkling Lake suggest that the concentration of CH_4 in the hypolimnion built up slowly during the summer stratification, reaching a maximum concentration in early November. After complete mixis occurred (by 17 November), CH_4 concentrations were greatly reduced throughout the water column.

Air–water exchange

Surface concentrations of CO_2 in bog lakes were above saturation at all times, resulting in CO_2 evasion throughout the open-water season (Fig. 5; Table 3). Estimated instantaneous (daily) rates surpassed 100 $mmol \cdot m^{-2} \cdot day^{-1}$ on several occasions (Fig. 5), reaching a maximum value of 232 $mmol \cdot m^{-2} \cdot day^{-1}$ in Trout Bog during autumn turnover. Seasonal patterns of CO_2 evasion differed between lakes, following patterns described for surface CO_2 concentrations. Spring turnover, when CO_2 accumulated under the ice was vented, was the period of most intense evasion in Crystal Bog, where evasion rates remained under 50 $mmol \cdot m^{-2} \cdot day^{-1}$ for the rest of the open-water season. In the deeper Trout Bog, in contrast, both spring and autumn turnover periods were important, and the highest evasion rates were recorded in autumn, partly as a result of high surface CO_2 concentrations (Fig. 1) and slightly higher wind speed.

Clear-water lakes were at times undersaturated, resulting in periods of CO_2 invasion. These were especially apparent in mesotrophic Sparkling Lake. However, evasion dominated during turnover periods, resulting in net evasion at the end of the open-water period (Table 3). Oligotrophic Crystal Lake was near equilibrium for most of the open-water season, but CO_2 evasion dominated during autumn turnover, resulting in net, but low, evasion at the end of the open-water period.

Bog lakes and clear-water lakes differed strongly in their mean daily exchange with the atmosphere during mixing periods (Table 2). During both spring and autumn turnover, mean daily CO_2 evasion rates were higher in bog lakes than in clear-water lakes. Compared with mean daily DIC loss rates (Table 2), evasion rates were higher than DIC loss during turnover periods in the bog lakes. By contrast, in the clear-water lakes, evasion rates were smaller than DIC loss rates in spring and of similar magnitude in autumn.

CH_4 evasion rates by diffusion were insignificant throughout most of the open-water season (Table 2), but high rates (over 20 $mmol \cdot m^{-2} \cdot day^{-1}$) were recorded during the spring (Crystal Bog) and autumn (Trout Bog) turnover periods (Fig. 6). CH_4 evasion from clear-water lakes was insignificant at all times, being always below 3 $mmol \cdot m^{-2} \cdot day^{-1}$.

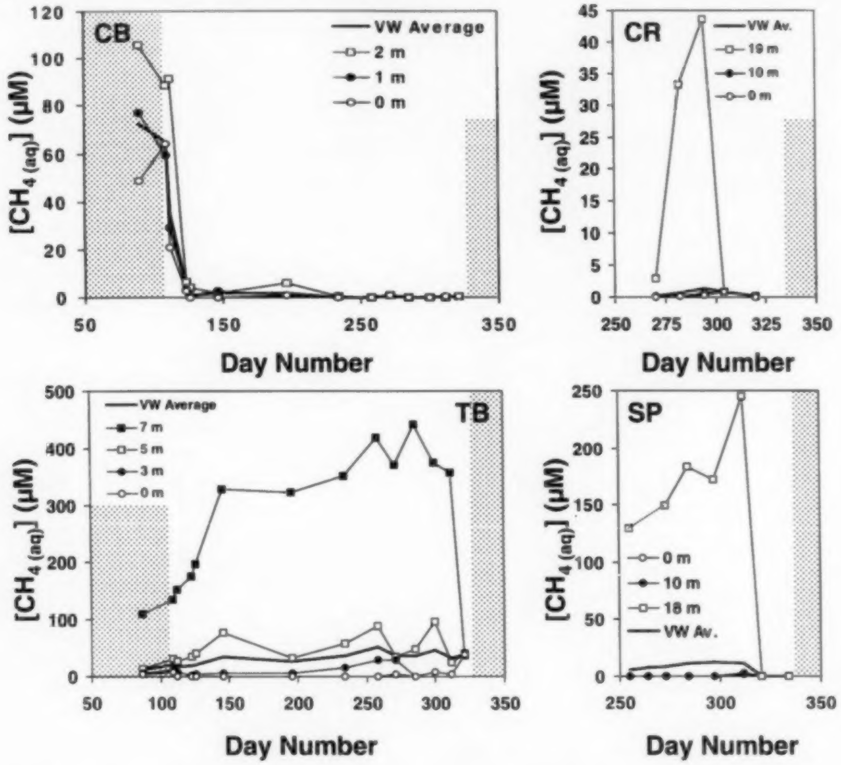
Discussion

Seasonal trends in CO_2 and CH_4 concentrations

A general model for the seasonal pattern of CO_2 and CH_4 in dimictic, clear-water lakes in the north temperate zone differentiates four distinct periods (Hesslein et al. 1991; Cole et al. 1994; Michmerhuizen and Striegl 1996; Cole and Caraco 1998): (*i*) accumulation of CO_2 and CH_4 under ice, with air–water exchange prevented by ice cover, (*ii*) high surface concentrations and rapid loss by evasion immediately after ice-off, (*iii*) decrease in CO_2 and CH_4 in surface waters after the onset of stratification, when CO_2 and CH_4 are removed from the epilimnion by diffusion to the atmosphere, phytoplankton uptake, and CH_4 oxidation, and CO_2 and CH_4 build up concurrently in the hypolimnion, and (*iv*) high surface concentrations of CO_2 and CH_4 during autumn turnover.

This pattern was generally followed by our lakes, but differences were found depending on lake type and productivity, lake morphometry, and the timing of mixing and stratification events. Sparkling and Crystal lakes, the two dimictic clear-water lakes, followed this general pattern. Crystal Lake, a small, oligotrophic, clear-water lake, had the lowest concentrations of all four lakes, with surface CO_2 concentrations close to equilibrium throughout most of the year. The fall increase in surface CO_2 was less pronounced in Crystal Lake than in Sparkling Lake. This diminished fall

Fig. 4. Concentration of CH_4 at selected depths for Crystal Bog (CB), Crystal Lake (CR), Trout Bog (TB), and Sparkling Lake (SP). Depth profiles for clear-water lakes were available only for autumn. The thick line in each graph is the volume-weighted average concentration of CO_2 in the lake. Ice cover is shown as shaded areas.



peak, relative to Sparkling Lake, is likely due to a smaller accumulation of CO_2 in the hypolimnion of the oligotrophic Crystal Lake during the stratified season.

Bog lakes had in common high concentrations of CO_2 and CH_4, owing to high loading rates of allochthonous carbon, and correspondingly showed high CO_2 and CH_4 evasion rates. However, the bog lakes differed in the timing and pattern of CO_2 and CH_4 accumulation and evasion due chiefly to differences in their morphometry. Trout Bog has a small area compared with its maximum depth (Table 1), is well sheltered, and mixing occurs only with difficulty. After ice-out, this lake did not mix completely, bottom waters remained hypoxic and soon became anoxic, and CO_2 and CH_4 buildup in the hypolimnion proceeded unimpeded throughout the short spring mixing period and into the summer. Autumn mixing, however, was longer lived, wind speed was slightly higher, and the lake mixed well, producing a high, sustained peak in surface CO_2 and CH_4 concentrations and, hence, high evasion to the atmosphere. Nonetheless, evasion rates were not sufficient to flush all the CO_2 and CH_4 that had accumulated throughout the open-water season.

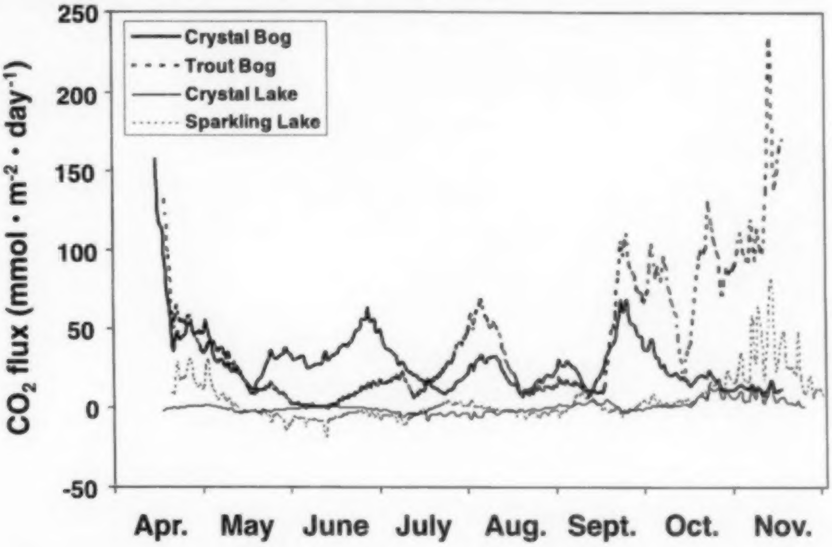
In sharp contrast, in shallow Crystal Bog, surface concentrations decreased sharply after ice-out and were maintained at moderately high levels throughout the rest of the open-water season by persistent entrainment of bottom waters. Therefore, no accumulation of CO_2 or CH_4 occurred to support a late summer peak.

Air–water fluxes

The estimation of gas transfer rates from wind speed is an active area of research, and several empirical relationships have been proposed (Liss and Merlivat 1986; Wanninkhof et al.1991; Wanninkhof 1992; MacIntyre et al. 1995; Wanninkhof and Knox 1996; Cole and Caraco 1998). We decided to use and Cole and Caraco's (1998) equation because it was developed for low-wind environments like our lakes. This equation gives higher values at wind speeds below 4 $m{\cdot}s^{-1}$ than other equations, thus recognizing that CO_2 evasion occurs even in the absence of wind if there is a strong gradient between surface waters and the atmosphere. Conversely, estimates for high wind speeds tend to be lower than those obtained using other equations. Thus, for Sparkling Lake, where wind speeds above 4 $m{\cdot}s^{-1}$ are not uncommon, CO_2 evasion was estimated at 1154 $mmol{\cdot}m^{-2}{\cdot}year^{-1}$ using Cole and Caraco's (1998) equation, but at 1970 $mmol{\cdot}m^{-2}{\cdot}year^{-1}$ if the equation proposed by Wanninkhof (1992) was used. Conversely, the estimate for Trout Bog, where low winds predominate, was 9963 $mmol{\cdot}m^{-2}{\cdot}year^{-1}$ using Cole and Caraco's (1998) equation, but only 5860 $mmol{\cdot}m^{-2}{\cdot}year^{-1}$ if Wanninkhof's (1992) equation was used.

All four lakes were net sources of CO_2 and CH_4 to the atmosphere (Table 3), but bog lakes and clear-water lakes differed enormously in their evasion rates. High concentrations in bog lakes supported high evasion rates, despite lower

Fig. 5. Estimated fluxes of CO_2 across the air–water interface at each of the lakes.



wind speeds than in clear-water lakes. In contrast, fluxes for Sparkling Lake were low. In Crystal Lake, fluxes were so low that, considering the uncertainties in their estimation, we must consider this lake to be at equilibrium on an annual basis.

Rates of CO_2 evasion were consistent with published values on other lakes. Instantaneous transfer rates varied in our lakes from negative values (–7.9 mmol·m^{-2}·day^{-1} in Crystal Lake) to maximum rates exceeding 200 mmol·m^{-2}·day^{-1}, whereas average daily rates varied from 0.3 to 42.7 mmol·m^{-2}·day^{-1}. Kling et al. (1991) recorded instantaneous rates between 5.5 and 59.8 mmol·m^{-2}·day^{-1} in a study of 25 lakes in Alaska. Hamilton et al. (1994) reported mean daily rates of 200 mmol·m^{-2}·day^{-1} for lowland fens in Hudson bay, Canada, but measured instantaneous rates were extremely high (exceeding 1000 mmol·m^{-2}·day^{-1}). Cole and Caraco (1998) estimated an annual flux of 2467 mmol·m^{-2}·year^{-1} for oligotrophic Mirror Lake, a value comparable with our estimate of 1128 mmol·m^{-2}·year^{-1} for Sparkling Lake.

Published estimates of CH_4 evasion rates are more scarce. Rudd and Hamilton (1978) reported an evasion rate of 0.34 mmol·m^{-2}·day^{-1} for artificially eutrophic Lake 227 at the Experimental Lakes Area in Canada. Kling et al. (1991) reported fluxes for Alaskan tundra ponds ranging from 0.08 to 1.02 mmol·m^{-2}·day^{-1} (mean 0.40 mmol·m^{-2}·day^{-1}). Hamilton et al. (1994) reported a mean daily rate of 10 mmol·m^{-2}·day^{-1} for 30 lowland fens, with maximum values exceeding 30 mmol·m^{-2}·day^{-1} on individual sampling dates. Mean daily values in our bog lakes were below 2 mmol·m^{-2}·day^{-1} (Table 3), but maximum instantaneous measurements exceeded 30 mmol·m^{-2}·day^{-1} after ice-off in Crystal Bog. Roulet et al. (1992) estimated the annual CH_4 flux of a beaver pond at 475 mmol·m^{-2}·year^{-1}, greater than our maximum value of 373 mmol·m^{-2}·year^{-1} (Trout Bog). Michmerhuizen and Striegl (1996) estimated potential CH_4 emissions in 19 north temperate lakes in North America based on winter CH_4 accumulation; their estimates ranged from 0.9 to 185 mmol·m^{-2}·year^{-1} and most likely underestimate annual fluxes. The importance of CH_4 emissions from wetland ponds is indisputable. Fluxes from clear-water lakes were significant, if modest.

Significance for lake carbon dynamics

On an annual scale, bog lakes and clear-water lakes contrasted strongly in the significance of CO_2 evasion to lake carbon dynamics. To assess this, we expressed CO_2 evasion as a percentage of the DIC mass in the lake at the start of the open-water period (Table 3). We found that in the clear-water lakes, CO_2 evasion was a small percentage (about 10%) of initial DIC, whereas in the bog lakes, CO_2 evasion represented a loss of DIC several times the initial mass in the lake (five times in Trout Bog and nine times in Crystal Bog). When CO_2 evasion is compared with total dissolved carbon (TDC), percentages are lower but still large for bog lakes. Also, CO_2 evasion is over 10 times larger than net DIC loss during the open-water period in the bog lakes, but only three times larger than net DIC loss in Sparkling Lake and smaller than net DIC loss in Crystal Lake (Table 3). These data suggest extremely fast carbon turnover rates in the bog lakes and imply the presence of large input rates to compensate for CO_2 losses to the atmosphere. If differences in DIC pools during the open-water season are taken into account, losses of CO_2 to the atmosphere must be compensated for by average daily inputs of 27.7 mmol·m^{-2}·day^{-1} in Crystal Bog and 44.7 mmol·m^{-2}·day^{-1} in Trout Bog. In Sparkling Lake, potential CO_2 inputs are estimated at 3.4 mmol·m^{-2}·day^{-1}, whereas in Crystal Lake, CO_2 evasion is smaller that the change in the pool of DIC, suggesting that there are net losses of carbon through other pathways, such as uptake and sedimentation and loss with groundwater.

Since CO_2 evasion occurs most intensely during mixing periods, we asked how CO_2 losses to the atmosphere compared with changes in the DIC and CO_2 pools during the same periods. Again, bog lakes differed strongly from clear-water lakes (Table 2). During mixis, CO_2 evasion in bog lakes was larger than the decrease in the pools of DIC and

Table 3. CO_2 and CH_4 fluxes and their relationship to the lake's carbon budget.

Lake	DIC initial (final), mmol·m^{-2}	TDC initial (final), mmol·m^{-2}	CO_2 flux annual (daily average), mmol·m^{-2}·year^{-1} (mmol·m^{-2}·day^{-1})	CO_2 evasion as % of initial DIC	CO_2 evasion as % of initial TDC	Annual CO_2 flux : ΔDIC ratio	CH_4 flux annual (daily average), mmol·m^{-2} (mmol·m^{-2}·day^{-1})	CH_4 evasion as % of initial TDC	Potential CH_4 production rates, mmol·m^{-2}·day^{-1}
Crystal Bog	715 (96)	1 956 (1182)	6701 (30.46)	937	343	10.83	273.6 (1.24)	14.0	0.66
Trout Bog	2 044 (1821)	11 235 (11 268)	9963 (45.7)	550	89	44.68	373.0 (1.71)	3.3	1.73
Crystal Lake	945 (525)	2 392 (2082)	89 (0.4)	9	4	0.21	27.3 (0.12)	1.1	0.15
Sparkling Lake	10 030 (9674)	13 191 (11 231)	1154 (5.0)	12	9	3.24	42.0 (0.18)	<0.1	0.13

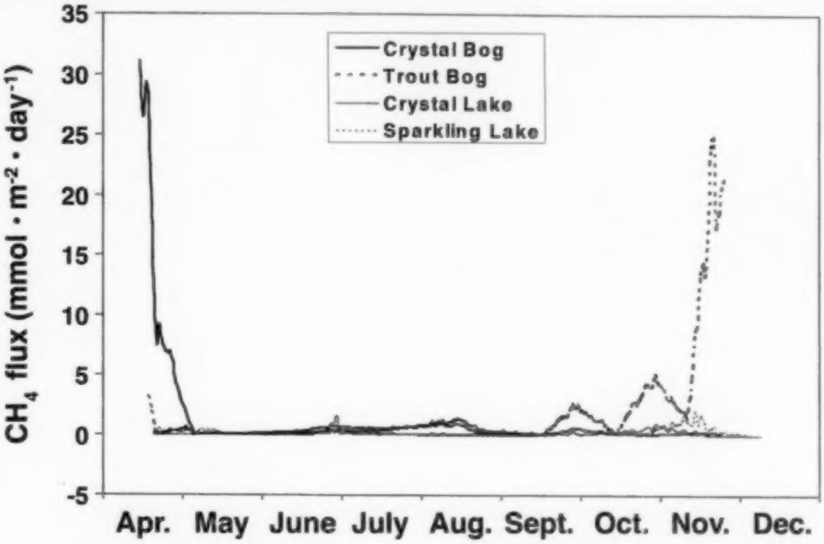
Note: The following values are given for CO_2: initial (last measurement before ice-out) and final (last measurement before ice-on) concentration of DIC and TDC, annual flux and average daily flux of CO_2, CO_2 evasion expressed as a percentage of initial DIC and of initial TDC, and the ratio between annual (=open-water season) CO_2 evasion and the net difference in DIC during the open-water season (ΔDIC). The following values are given for CH_4: annual and average daily flux of CH_4, CH_4 evasion as a percentage of initial TDC, and potential CH_4 production rates (calculated as total flux minus the CH_4 pool change during the ice-free period divided by the duration of the ice-free period in days).

CO_2, an indication that CO_2 inputs increased during the same period. In clear-water lakes, by contrast, CO_2 evasion was smaller that the decrease in DIC and CO_2, and in Crystal Lake, CO_2 invasion occurred in spring. Therefore, other pathways of CO_2 consumption, such as photosynthetic uptake, prevail in clear-water lakes. During the autumn mixing period, a different pattern emerges. In clear-water lakes, CO_2 evasion is of the same order of magnitude as changes in DIC and CO_2 pools. In the bog lakes, in contrast, our data suggest that CO_2 inputs are large at the same time that evasion is high. This is particularly clear in Trout Bog, where the pools of DIC and CO_2 increased as the lake was losing CO_2 to the atmosphere during the period of thermocline erosion. Full turnover was not detected until only 5 days before ice-on, precluding complete flushing of CO_2 in the lake.

How can we explain the large CO_2 inputs necessary to account for CO_2 evasion? Inputs of CO_2 may occur in two ways in seepage lakes (Hope et al. 1996; Cole and Caraco 1998): (*i*) net in-lake CO_2 production (net heterotrophy) and (*ii*) CO_2 input with groundwater. A third possible mechanism (alkalinity consumption) must be ruled out in our lake district due to the low alkalinity of lake water. In-lake production appears to be insufficient to explain the evasion rates observed in our lakes, especially in bog lakes. Available estimates of production to respiration rates suggest that in-lake CO_2 production may be insufficient to explain CO_2 inputs (Hope et al. 1996). Clear-water lakes had low primary productivity (*P*), low respiration rates (*R*), and high *P*/*R* ratios (e.g., 0.83 in neighboring Trout Lake). Crystal Bog and Trout Bog are more productive, but also have higher respiration rates, resulting in lower *P*/*R* ratios. For example, Hope et al.'s (1996) measurements of in-lake CO_2 production in Trout Bog in July 1994 yielded 5.7 mmol·m^{-2}·day^{-1}, very far from the average 44.7 mmol·m^{-2}·day^{-1} necessary to compensate for CO_2 evasion, although CO_2 production should be higher at other times, especially during autumn turnover. In contrast, evidence suggests that large inputs of allochthonous CO_2 may be supporting CO_2 evasion in our lakes. The high concentrations of CO_2 in the surrounding bogs suggest that high CO_2 inputs from these may occur. Likewise, high concentrations of CO_2 in groundwater suggest that CO_2 inputs, rather than in-lake production of CO_2, are important also in the clear-water lakes. For a set of 27 lakes in this area, Hope et al. (1996) found a strong relationship between CO_2 concentration (hence, CO_2 evasion) and DOC and between DOC and the drainage ratio and wetland extent. These relationships suggest large inputs of allochthonous carbon.

The significance of CH_4 evasion also contrasted strongly between clear-water lakes, where it is negligible, and bog lakes, where it is higher (Table 3). Compared with CO_2 evasion rates, CH_4 evasion rates represent about 4% of total carbon efflux to the atmosphere (but 23% in Crystal Lake). We can estimate potential CH_4 production rates by taking into account the changes in the CH_4 pool during the open-water season (Table 3). Values ranged from 0.13 mmol·m^{-2}·day^{-1} in Sparkling Lake to 1.38 mmol·m^{-2}·day^{-1} in Trout Bog. Since these values do not take into account CH_4 losses by oxidation, actual CH_4 production rates must be higher. In Lake 227 of the Experimental Lakes Area, Rudd and Hamilton (1978) found that CH_4 oxidation was about twice as

Fig. 6. Estimated fluxes of CH_4 across the air–water interface at each of the lakes.



large as CH_4 evasion during the summer. If the same were true for our lakes, CH_4 production rates would be larger than our estimates suggest.

Conclusions

Differences in seasonal dynamics were related to differences in lake morphometry and exposure to the atmosphere. Bog lakes were small and sheltered (wind speed was lower than in the two clear-water lakes), but Trout Bog was deep (relative to surface area) and stratified strongly enough that most of the CO_2 and CH_4 stored during the ice-covered period remained trapped in the hypolimnion throughout the summer. In contrast, Crystal Bog was shallow and stratified weakly, thus venting CO_2 and CH_4 throughout the summer season. Clear-water lakes were larger and more exposed to the atmosphere (wind speed was consistently higher than in bog lakes) and stratified stably, venting CO_2 and CH_4 significantly during turnover periods.

The magnitude of CO_2 and CH_4 concentrations and fluxes between air and water differed tremendously between bog lakes and clear-water lakes. Driven by high CO_2 and CH_4 concentrations, most likely the result of allochthonous inputs, fluxes of those gases to the atmosphere were high in bog lakes. Compared with changes in the carbon pools in the lake, the fluxes observed suggest fast carbon turnover rates in bog lakes. In contrast, low surface concentrations in clear-water lakes, with undersaturation common during the summer months, resulted in modest evasion from clear-water lakes. Nonetheless, net evasion was observed in all lakes, and at the landscape scale, evasion from larger clear-water lakes might be more important due to the higher area that they cover.

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Impact of age-0 bluefish (*Pomatomus saltatrix*) predation on age-0 fishes in the Hudson River estuary: evidence for density-dependent loss of juvenile striped bass (*Morone saxatilis*)

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Abstract: We measured bluefish (*Pomatomus saltatrix*) weights, densities, and prey sizes during the summers of 1992 and 1993 and diets over a 4-year period (1990–1993) in the Hudson River estuary. This information was used to estimate the loss of young-of-the-year (YOY) striped bass (*Morone saxatilis*) resulting from YOY bluefish predation. We then compared this predation mortality with the total loss of striped bass in the system. Data from sampling surveys conducted since the mid-1970's were used to examine relationships between bluefish abundance and striped bass recruitment levels. YOY striped bass, bay anchovy (*Anchoa mitchilli*), Atlantic silverside (*Menidia menidia*), and *Alosa* spp. dominated YOY bluefish diets. There were ontogenetic and interannual differences in YOY bluefish diets. Bluefish avoided striped bass at low densities but selected for them at high densities, suggesting a density-dependent feeding response. In the early summer of 1993, bluefish predation accounted for 50–100% of the total estimated loss of YOY striped bass. A significant negative correlation exists between the relative magnitude of striped bass recruitment and bluefish abundance. We conclude that YOY bluefish are important predators of estuarine fish and can have a substantial impact on their recruitment.

Résumé : Nous avons mesuré le poids et la densité de tassergals (*Pomatomus saltatrix*) et la taille de leurs proies durant les étés 1992 et 1993, ainsi que leur régime alimentaire sur une période de 4 ans (1990–1993) dans l'estuaire de l'Hudson. Ces données ont été utilisées pour estimer la diminution de l'abondance des jeunes bars rayés (*Morone saxatilis*) de l'année due à la prédation par les jeunes tassergals de l'année. Nous avons ensuite comparé cette mortalité par prédation à la diminution totale de l'abondance du bar rayé dans le milieu. Nous avons utilisé les données de campagnes d'échantillonnage menées depuis le milieu des années 70 pour examiner la relation entre l'abondance du tassergal et les niveaux de recrutement du bar rayé. Les jeunes bars rayés de l'année, les anchois américains (*Anchoa mitchilli*), les capucettes (*Menidia menidia*) et les *Alosa* spp. constituaient la plus grande partie du régime alimentaire des jeunes tassergals de l'année. Il y avait des différences ontogénétiques et interannuelles dans les régimes alimentaires des jeunes tassergals de l'année. Les tassergals évitaient les bars rayés quand ces derniers étaient présents en faible densité, mais s'en nourrissaient quand leur densité était élevée; il y aurait donc chez les tassergals une réponse alimentaire dépendante de la densité. Au début de l'été de 1993, 50 à 100% de la diminution totale estimée de l'abondance des jeunes bars rayés de l'année était attribuable à la prédation par les tassergals. Une corrélation négative significative existe entre l'importance relative du recrutement des bars rayés et l'abondance des tassergals. Nous concluons que les jeunes tassergals de l'année sont d'importants prédateurs des poissons estuariens et peuvent avoir un impact substantiel sur leur recrutement.

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Introduction

Determination of the factors that affect recruitment of marine fish populations is an area of intense study. Biotic (food availability, predation, etc.) and abiotic factors (temperature, loss to advection, etc.) have both been shown to influence recruitment (for a review, see Leggett and DeBlois 1994), necessitating a multifactorial approach to understanding recruitment variation (Fogarty et al. 1991; Leggett and DeBlois 1994). Much research has focused on factors affecting egg and larval stages; however, there has been a resurgence of interest in processes affecting juvenile mortality (Hixon 1991; Walters and Juanes 1993; Bailey 1994).

In freshwater systems the effects of predation on juvenile fish are well documented. For example, piscivores directly affect recruitment in many freshwater fishes (Nielsen 1980; Tonn et al. 1992). Evidence of indirect effects of predation on prey fish populations in freshwater systems also exists (Kerfoot and Sih 1987; He and Wright 1992).

Far fewer studies have examined the effects of piscivores in open marine systems such as estuaries and the coastal ocean. Campbell (1979) and Sykes and Manooch (1979) reviewed piscine predation studies in rivers and estuaries, respectively. No studies found conclusive evidence of a direct predatory effect on prey fish populations. More recently, Rieman et al. (1991) determined the amount of juvenile salmonid (*Oncorhynchus* spp.) losses that resulted from fish predation in the Columbia River. They found that mortality from fish predators (mostly northern squawfish (*Ptychocheilus oregonensis*)) accounted for a previously unexplained source of mortality during the out-migration of juvenile salmonids. Experiments designed to investigate the effects of piscivory on estuarine juvenile fish populations have been conducted in salt pond or mesocosm enclosures (Wright et al. 1993; Gleason and Bengtson 1996*a*), but direct in situ measurements of the impact of piscivores on juvenile prey fish populations in estuaries are lacking (but for a coastal nursery example, see van der Veer and Bergman 1987).

The bluefish (*Pomatomus saltatrix*) is a marine piscivore of circumglobal distribution that occurs nearshore in its juvenile stage (Juanes et al. 1996). In the western Atlantic, spawning occurs offshore over the continental shelf and juveniles migrate abruptly into U.S. east coast estuaries at ~60 mm fork length (Kendall and Walford 1979; Nyman and Conover 1988; McBride and Conover 1991). Bluefish spawned in the South Atlantic Bight in the spring are advected northward in waters associated with the Gulf Stream (Hare and Cowen 1996) and move into New York – New Jersey estuaries in June (Nyman and Conover 1988; McBride and Conover 1991). A second wave of recruits consisting of summer-spawned fish recruit into estuaries in middle to late summer (Nyman and Conover 1988; McBride and Conover 1991). The offshore to inshore habitat shift coincides with a feeding shift from zooplanktivory to piscivory (Marks and Conover 1993).

A large number of studies have determined the diet of age-0 bluefish along the east coast of the United States (see references in Buckel and Conover 1997). Age-0 bluefish prey includes the larval and juvenile stages of a variety of fish species. In the Hudson River estuary, Juanes et al. (1993) found that juvenile bluefish prey includes several important anadromous species such as American shad (*Alosa sapidissima*) and striped bass (*Morone saxatilis*). Friedland et al. (1988) found year-to-year differences in diets of age-0 bluefish in a New Jersey marine embayment. Bluefish are believed to be opportunistic predators and appear to feed in relation to prey abundance (Juanes et al. 1993).

Here, we present diet and prey selectivity data from bluefish collected over a 4-year period (1990–1993) throughout a large portion of the Hudson River estuary (24 km). Using field estimates of bluefish consumption rates (Buckel and Conover 1997), prey sizes, and bluefish and prey densities, the loss of age-0 striped bass resulting from age-0 bluefish predation in the Hudson River estuary is estimated. This predation mortality is compared with the total loss of striped bass in the system. Additionally, data from sampling surveys conducted since the mid-1970's were used to examine relationships between striped bass recruitment and bluefish abundance.

Materials and methods

Study area and fish collections

The lower Hudson River is one of several major estuaries along the east coast of the United States (41°00′N, 73°54′W). It functions as a nursery for a diverse ichthyofaunal community consisting of several anadromous (clupeid and percichthyid) and estuarine (engraulid and atherinid) fishes (Beebe and Savidge 1988).

There were two sources of bluefish from the Hudson for diet analysis. The first included bluefish specimens collected by the New York State Department of Environmental Conservation's (NYDEC) juvenile striped bass sampling program over a 4-year period (1990–1993). This survey has been conducted since 1976 and involves nine biweekly samples of 25 stations from mid-July through early November. Sampling gear consists of a 61 × 3 m beach seine (13-mm-mesh wings and a 6-mm-mesh bag) deployed by boat. The survey samples a 24-km section of the lower Hudson River estuary from river kilometre ~39 to 63 (see fig. 1 in Juanes et al. 1993). This area of the estuary contains the majority of the summer age-0 striped bass population (Versar, Inc. 1988; available from Versar, Inc., 9200 Rumsey Road, Columbia, MD 21045, U.S.A.).

The second source of bluefish (early field collection, EF) was from biweekly collections at a subset (eight sites) of the NYDEC beach seine locations. These collections were performed from mid-June to early July 1992 and 1993 to determine the timing of first bluefish recruitment to the lower Hudson and diet during this time period. Collections were with identical gear as described above.

In both surveys, one seine haul was conducted at each station during daylight hours. This is a time period when bluefish are known to feed (Buckel and Conover 1997). The contents of the net were identified, enumerated, and put back into the river with the exception of bluefish and subsamples of potential prey, which were preserved in 10% buffered formalin.

Diet

Diets of bluefish captured throughout the sampling seasons of 1990–1993 were evaluated. Bluefish were measured for total length (TL) and their stomachs extracted. Stomach contents of bluefish were identified to the lowest possible taxon, enumerated, blotted dry, weighed (± 0.01 g), and measured (TL, ± 1.0 mm; eye diameter, ± 0.1 mm; caudal peduncle depth, ± 0.1 mm). Regressions relating prey eye diameter and caudal peduncle depth to TL were used to estimate prey TL of partially eaten prey (see Scharf et al. 1997). A reference collection of Hudson River fish species (whole fish, scales (see Lassiter 1962), and bones) was used to aid in identification of digested prey. Differences in dentition and anal fin morphologies were used to aid in identification of the congeners white perch (*Morone americana*) and striped bass (Fritzsche and Johnson 1980; Waldman 1986). Differences in mouth morphologies were used to aid in the identification of *Alosa* spp.

Each beach seine haul provided us with a group or "cluster" of bluefish for a given station as in a cluster sampling design (Cochran 1977). The mean and variance of diet indices were calculated with cluster sampling estimators developed at the Northeast Fisheries Science Center, National Marine Fisheries Service, Woods Hole, Mass. (M.J. Fogarty, University of Maryland System, Chesapeake Biological Laboratory, Solomons, MD 20688, U.S.A., unpublished data). For each sampling date, an estimate of the mean proportion of stomachs (frequency of occurrence) that contain prey type k (F_k) was calculated as

$$F_k = \frac{\sum_{i=1}^{n} M_i p_{ik}}{\sum M_i}$$

where

$$p_{ik} = \frac{m_{ik}}{m_i}$$

and where n is the number of seine hauls containing bluefish, M_i is the number of bluefish captured in a beach seine haul at station i, m_i is the number of bluefish in a subsample of the fish from station i that were analyzed for diet, and m_{ik} is the number of bluefish in the subsample with food type k at station i. The estimator of variance for F_k is

$$v(F_k) = \frac{1}{n\bar{M}^2} \frac{\sum_{i=1}^{n} M_i^2 (p_{ik} - F_k)^2}{n-1}$$

where $\bar{M}$ is the average number of fish in a seine haul on that date. The estimate of the mean proportional contribution of a prey type by weight (W_k) was calculated as

$$W_k = \frac{\sum_{i=1}^{n} M_i q_{ik}}{\sum M_i}$$

where

$$q_{ik} = \frac{w_{ik}}{w_i}$$

and where w_i is the total weight of all identifiable prey in a subsample of the fish from station i that was analyzed for diet and w_{ik} is the weight of prey type k in the subsample at station i. The estimator of variance for W_k is

$$v(W_k) = \frac{1}{n\bar{M}^2} \frac{\sum_{i=1}^{n} M_i^2 (q_{ik} - W_k)^2}{n-1}.$$

Prey type selectivity

Feeding selectivity of juvenile bluefish in the Hudson River estuary was determined from relative abundance of prey in bluefish stomachs and beach seine catches (see Fig. 1). Four fish prey categories were examined: striped bass, clupeids (American shad and blueback herring (*Alosa aestivalis*)), Atlantic silverside (*Menidia menidia*), and other fish (white perch, *Fundulus* spp., Atlantic menhaden (*Brevoortia tyrannus*), hogchoker (*Trinectes maculatus*), and Atlantic tomcod (*Microgadus tomcod*)). These prey, along with bay anchovy (*Anchoa mitchilli*), made up 90–98% of the potential fish prey collected during beach seine collections during all 4 years of collection (1990–1993). Bluefish selectivity for bay anchovy was not calculated because the larval and early juvenile stages that were often found in bluefish guts were not collected efficiently in the beach seine gear. Chesson's (1978) index was used to determine bluefish prey preference as

$$\alpha_i = \frac{r_i/p_i}{\sum_{j=1}^{m} r_j/p_j}, \; i = 1, \ldots, m$$

where α_i is the selectivity for prey type i for a group of bluefish from a given beach seine haul, r_i is the relative abundance of prey type i for the group of bluefish stomachs in a given beach seine haul, p_i is the relative abundance of prey type i in the environment calculated from the combined seine catches in which bluefish stomachs were collected on a given date, and m is the number of prey types available ($m = 4$). Values of r_i for prey that were not identified to the species level (e.g., *Morone* spp.) were partitioned to identifiable categories based on the relative occurrence of the identifiable species making up each category. Values of α_i were calculated for each beach seine haul. The mean α_i for each date was calculated using the cluster sampling estimators described above. The variance was not estimated because of the lack of independence between estimates of α_i on a given date — an identical value of p_i was used for each beach seine haul estimate of α_i. For statistical purposes, a unique value of p_i for each seine haul estimate of α_i would have been preferred, but the absence of prey species from individual beach seine hauls precluded this technique (zeros often occurred in the denominator). Pooling prey across seine hauls to develop estimates of relative abundance for the four prey types on each date may be more ecologically realistic given the movements of bluefish in the system. Random feeding occurs when $\alpha_i = m^{-1}$ (0.25); values of $\alpha_i > 0.25$ or $\alpha_i < 0.25$ represent "selection" and "avoidance" of prey, respectively. Values of α_i can be compared among dates even if the relative abundances of prey types differ (Chesson 1983).

A nonparametric Kruskal–Wallis test was used to examine the influence of year on prey selection of striped bass; within-year variation could not be measured given the different sampling dates from one year to the next. To determine if bluefish feed disproportionately on prey as prey density changes, a Pearson correlation coefficient was calculated between striped bass α_i values and striped bass catch-per-unit-effort (CPUE).

Prey loss calculations

Daily loss of juvenile striped bass to bluefish predation was calculated from bluefish density (number per 100 m^2, D ± SE), weight (grams, W ± SE), daily ration (grams per gram per day, C ± SE), the proportion of striped bass by weight in bluefish diet (P ± SE), and striped bass sizes eaten by bluefish (grams, S ± SE). Values for D, W, P, and S are from EF/NYDEC collections. Values of D were calculated from bluefish CPUE data (Table 1) assuming that the 61-m seine sampled a 500-m^2 area (McBride et al. 1995). Bluefish C values (Table 2) were measured in the lower Hudson River during the summers of 1992 and 1993 by sampling bluefish gut fullness levels over a diel period (Buckel and Conover 1997); additional bluefish W (Table 1) and S data came from these diel sampling collections.

Equations to estimate striped bass lengths consumed by bluefish were developed using sizes of striped bass retrieved from bluefish guts throughout the summers of 1992 and 1993 (1992: striped bass TL eaten (millimetres) = 0.682·(day) + 3.573, $r^2 = 0.70$, $n = 66$; 1993: striped bass TL eaten (millimetres) = 0.965·(day) − 9.081, $r^2 = 0.80$, $n = 135$; day 0 = June 1); these length equations were then substituted into the following equation to predict striped bass prey weights consumed by bluefish on a given date (S (grams) = $7.0 \times 10^{-6} \cdot \mathrm{TL}^{3.06}$, $r^2 = 0.99$, $n = 246$). The delta method (Seber 1973) was used to approximate a standard error for the resulting 1992 and 1993 functions describing prey weight as a function of date.

In order to obtain a daily value of D, W (log$_e$ transformed to linearize), C, and P, linear interpolations were used between actual estimates. Daily values of SE's for these quantities were estimated from the general relationship for the variance of a linear function of two independent random variables (Hogg and Craig 1970). The number of striped bass eaten by bluefish per 100 m^2 (± SE) of the

Table 1. Weight and CPUE of YOY bluefish collected with a 61-m beach seine in the lower Hudson River estuary for 1992 and 1993.

		Weight (g)		CPUE	
Date	Survey	No. of fish	Mean (SD)	No. of hauls	Mean (SD)
1992					
June 18	EF	1	1.54	3	0.33 (0.58)
June 26	EF	20	2.39 (0.41)	8	2.25 (1.83)
July 7	EF	85	3.04 (1.24)	8	6.57 (5.35)
July 16–17	24-h	179	4.48 (2.43)	na	na
July 22–23	NYDEC	90	9.26 (5.95)	25	3.76 (3.77)
July 28–29	24-h	85	11.30 (6.40)	na	na
August 5–6	NYDEC	47	18.73 (10.46)	25	2.00 (1.94)
August 13–14	24-h	119	26.79 (14.18)	na	na
August 18–19	NYDEC	17	21.04 (9.90)	25	1.92 (2.45)
August 26–27	24-h	85	41.16 (25.49)	na	na
September 1–2	NYDEC	22	45.40 (18.92)	25	0.92 (1.29)
September 15–16	NYDEC	8	54.88 (24.05)	25	0.52 (1.12)
September 19–20	24-h	96	43.71 (19.73)	na	na
October 1–2	NYDEC	1	68.71	25	0.04 (0.20)
1993					
June 16	EF	na	na	8	0
June 30	EF	57	3.87 (1.43)	7	8.14 (1.86)
July 7–8	24-h	248	4.96 (1.60)	na	na
July 13–14	NYDEC	117	6.17 (6.55)	25	4.52 (4.73)
July 20–21	24-h	136	8.96 (5.94)	na	na
July 28–29	NYDEC	73	21.55 (12.44)	25	3.92 (3.98)
August 4–5	24-h	119	19.84 (10.24)	na	na
August 10–11	NYDEC	161	22.87 (11.90)	25	5.26 (8.48)
August 11–12	24-h	47	23.29 (9.64)	na	na
August 18–19	24-h	130	33.30 (18.02)	na	na
August 24–25	NYDEC	23	47.31 (31.98)	25	1.64 (1.98)
September 7–8	NYDEC	34	84.57 (35.15)	25	2.00 (4.80)
September 11–12	24-h	23	75.67 (42.16)	na	na
September 29–30	NYDEC	2	119.66 (7.25)	25	0.20 (0.41)

Note: See text for description of the NYDEC and EF surveys. Weight data from 24-h surveys are from Buckel and Conover (1997). na, not applicable.

nearshore per day was calculated in three steps. First, nearshore bluefish biomass (B ± SE) was calculated as

$$B\,(\mathrm{g}\cdot 100\ \mathrm{m}^{-2}) = D\,(\mathrm{no.}\cdot 100\ \mathrm{m}^{-2}) \times W\,(\mathrm{g}).$$

Second, the grams of striped bass consumed by bluefish per 100 m^2 per day (SBC ± SE) was calculated as

$$\mathrm{SBC}\,(\mathrm{g}\cdot 100\ \mathrm{m}^{-2}\cdot \mathrm{day}^{-1}) = B\,(\mathrm{g}\cdot 100\ \mathrm{m}^{-2}) \times C\,(\mathrm{g}\cdot \mathrm{g}^{-1}\cdot \mathrm{day}^{-1}) \times P.$$

Third, the number of striped bass consumed by bluefish per 100 m^2 per day (N ± SE) was calculated as

$$N\,(\mathrm{no.}\cdot 100\ \mathrm{m}^{-2}\cdot \mathrm{day}^{-1}) = \mathrm{SBC}\,(\mathrm{g}\cdot 100\ \mathrm{m}^{-2}\cdot \mathrm{day}^{-1})/S\,(\mathrm{g}).$$

Estimates of N were calculated from the date that bluefish were first captured in substantial numbers (June 26, 1992, and June 30, 1993) until bluefish densities declined to a low level (September). Standard errors of products or quotients in the above equations were approximated using the delta method (Seber 1973). The cumulative consumption of striped bass was calculated for N and ± 1 SE of N. These cumulative consumption curves were compared with beach seine striped bass density estimates (number per 100 m^2) from the EF/NYDEC survey and with an independent estimate of striped bass abundance (beach seine CPUE) from an identical area of the Hudson River conducted by the Hudson River Estuary Monitoring Program (HREMP) (described below).

Striped bass mortality resulting from bluefish predation (M_{pred_i}, per day) was calculated for each interval length i between striped bass sampling dates as

$$M_{\mathrm{pred}_i} = \frac{N_i}{\overline{X}_i}$$

where N_i is the total number of striped bass consumed by bluefish per 100 m^2 for the interval i and $\overline{X}_i$ is the geometric mean number of striped bass per 100 m^2 (EF/NYDEC) over that same time interval. The intervals used to estimate total loss and predation loss of striped bass were based on striped bass sampling dates. These calculations were also performed using the sum of striped bass consumed at ± 1 SE of N_i. Estimates of striped bass predation mortality (M_{pred_i}) were divided by the interval length i in days to get daily estimates of predation mortality. Values of daily striped bass mortality resulting from bluefish predation were compared with estimates of total striped bass loss. Striped bass loss (mortality plus the sum of emigration and immigration) was determined from EF/NYDEC data from the end of June until early August using

Table 2. Estimated YOY bluefish consumption rates from Buckel and Conover (1997).

Date	Consumption rate ($g \cdot g^{-1} \cdot day^{-1} \cdot 100$)	SE
1992		
July 16–17	22.20	5.71
July 28–29	17.45	3.70
August 13–14	14.73	2.67
August 26–27	12.62	2.82
September 19–20	7.34	3.00
1993		
July 7–8	10.05	1.92
July 20–21	14.67	3.40
August 4–5	13.90	4.77
August 18–19	10.06	1.94

catch curve analysis; the instantaneous mortality rate (Z, per day) was calculated as the slope of the linear regression of $\log_e$(CPUE) against day (Ricker 1975). Daily conditional mortality rates (percent per day) were calculated as $1 - e^{-M}$ and $1 - e^{-Z}$.

Relationship between bluefish and striped bass abundance

HREMP has conducted an extensive ichthyoplankton and juvenile fish sampling survey since 1974 (for methodologies, see Dey 1981; Barnthouse et al. 1988; Pace et al. 1993). The program is considered among the most intensive long-term studies of fish populations ever conducted (Pace et al. 1993). A Pearson correlation coefficient was calculated between the NYDEC young-of-the-year (YOY) striped bass index (geometric mean) measured in the lower Hudson River estuary and HREMP's striped bass post-yolk-sac larvae (PYSL) index. There were 18 years that had coincident data (1977–1978, 1980–1995) from the two surveys.

An annual YOY bluefish index from the HREMP beach seine survey from 1974 to 1995 was calculated. The index is the arithmetic mean bluefish CPUE for the Tappan Zee and Croton-Haverstraw regions of the lower Hudson River (river kilometres 39–63) averaged for the month of July. This area of the Hudson is identical to the area sampled by the NYDEC YOY striped bass survey. The bluefish index was determined for those years in which there was at least 2 weeks of sampling in the month of July. This excluded 1981–1984. A Pearson correlation coefficient between the NYDEC YOY striped bass index and the YOY bluefish index was calculated for the 14 years of coincident data.

Results

Diet

The diets of 1585 spring-spawned and 197 summer-spawned bluefish (cohort determined from size differences) were examined from fish collected in the Hudson River during the summers of 1990–1993 (1990: 575 spring, 99 summer; 1991: 258, 25; 1992: 290, 60; 1993: 462, 13). In all four years, the dominant prey type of spring- and summer-spawned bluefish was teleost fish.

Spring-spawned bluefish had not recruited to the lower Hudson River during the mid-June EF collection in 1992 or 1993 (one bluefish was captured on June 18, 1992; no bluefish were captured on June 16, 1993; see Table 1). However, in both years, bluefish were captured in large numbers by the end of June. Fish prey was the largest diet component of recently recruited bluefish (see Buckel (1997) for values of bluefish diet by percent; the diet data presented in Fig. 1 have been normalized because percentages from cluster estimators do not add up to 100%). The dominant prey of bluefish captured in EF collections was striped bass in both 1992 and 1993 (Fig. 1; Table 3). Other bluefish prey during this time period included *Alosa* spp., blueback herring, bay anchovy, and Atlantic silverside. Invertebrate prey, crab zoea (mostly *Rhithropanopus harisii*) and calanoid copepods, were also an important part of the diet of recently recruited spring-spawned bluefish (Fig. 1).

The dominant prey of spring-spawned bluefish collected from mid-July to September–October in the four years studied (1990–1993) were bay anchovy, striped bass, Atlantic silverside, American shad, and blueback herring (Fig. 1). The importance of these prey varied from year to year. Bay anchovy was the most consistently taken prey for all four years (Fig. 1). Striped bass, white perch, and *Morone* spp. were important dietary items of spring-spawned bluefish in 1990, 1992, and 1993 but not in 1991. Atlantic silverside and clupeids were more variable. The Atlantic silverside was not an important prey in 1990 and was a dominant part of the diet on only one date in 1992 (Fig. 1). However, it was a major prey item of spring-spawned bluefish in 1991 and 1993. Clupeids (American shad, blueback herring, alewife (*Alosa pseodoharengus*), and *Alosa* spp.) were nearly absent in bluefish stomachs in 1993, intermediate in occurrence in 1991, but dominated the diet on some collection dates in 1990 and 1992 (Fig. 1).

Invertebrate prey of spring-spawned bluefish collected from mid-July to September–October included crab zoea (mostly *R. harisii*), unidentified copepods, unidentified isopods, unidentified amphipods, blue crab (*Callinectes sapidus*), sand shrimp (*Crangon* spp.), and grass shrimp (*Palaemonetes* spp.). These invertebrate prey were usually rare in bluefish stomachs with the exception of some dates where zoea and copepods and sand and grass shrimp were relatively important dietary items.

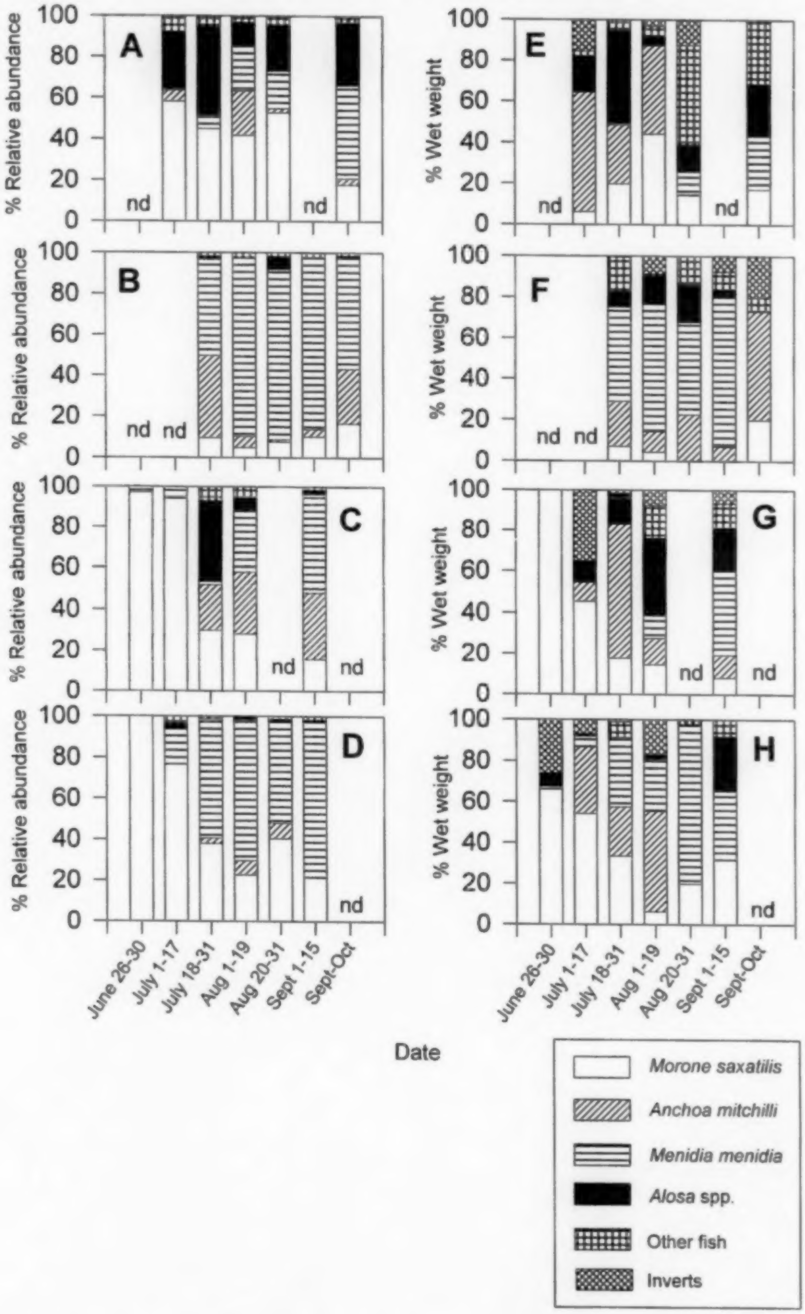
The diets of summer-spawned bluefish were dominated by bay anchovy in all four years (see Buckel 1997). Striped bass were not an important prey of summer-spawned bluefish (Table 3).

Prey type selectivity

Selectivity values (Chesson's α) indicated that spring-spawned bluefish showed considerable variation in prey preference. Bluefish often fed randomly with respect to prey abundance but, on occasion, also appeared to select or avoid prey (Table 4). There were both seasonal and interannual differences in selectivity for a given prey type. For example, there was a significant (Kruskal–Wallis ANOVA, $\chi^2 = 8.53$, df = 3, $p = 0.036$) interannual difference in selection of striped bass by bluefish. Bluefish often fed randomly with respect to striped bass abundance in 1990 and 1993 while avoiding them in 1991 (Table 4). In June and July of 1992 and 1993, bluefish showed positive selection for striped bass but then fed randomly or avoided them in later months.

Bluefish preference for striped bass was positively correlated with EF/NYDEC-measured striped bass CPUE ($r = 0.73$, $p = 0.001$) (Fig. 2). Hence, bluefish appear to select striped bass as prey when they are abundant.

Fig. 1. Percent relative abundance of potential YOY bluefish prey from beach seine CPUE data during EF/NYDEC sampling in the Hudson River estuary for (A) 1990, (B) 1991, (C) 1992, and (D) 1993 and the mean percent wet weight contribution of prey to YOY bluefish diet in the Hudson River estuary estimated from a cluster sampling design (see Buckel (1997) for cluster estimator percentages; values are normalized to 100%) for (E) 1990, (F) 1991, (G) 1992, and (H) 1993. nd, no data.



Bluefish showed a preference for clupeids on at least one date in all four years. Bluefish mostly selected for Atlantic silverside in 1991 and 1993 while avoiding them on most dates in 1990 and 1992.

Prey loss calculations

Estimates of mean daily predation loss were made for three intervals in 1992 and 1993. In 1992, the loss of striped bass during June 26 – July 7, July 7–22, and July 22 –

Table 3. Proportion of striped bass in spring- and summer-spawned bluefish diets during 1990–1993 beach seine collections in the Hudson River estuary.

	1990		1991		1992		1993	
Date	%*F*	%*W*	%*F*	%*W*	%*F*	%*W*	%*F*	%*W*
Spring-spawned bluefish								
June 26–30	nd	nd	nd	nd	9.5 (7.4)	28.6 (22.1)	17.5 (4.9)	56.3 (15.2)
July 1–17	4.6 (2.3)	6.0 (3.7)	nd	nd	9.5 (4.7)	37.5 (21.0)	18.8 (5.0)	51.0 (13.7)
July 18–31	8.2 (3.7)	17.7 (7.8)	3.4 (2.2)	6.7 (5.6)	5.5 (3.2)	15.5 (10.0)	11.3 (5.2)	25.6 (17.6)
August 1–19	4.3 (3.2)	20.5 (18.6)	1.5 (2.2)	3.8 (5.5)	6.3 (7.0)	12.3 (14.7)	1.2 (1.3)	3.9 (4.7)
August 20–31	1.5 (1.9)	4.9 (6.7)	0	0	6.3 (7.0)	12.3 (14.7)	9.1 (22.5)	17.1 (42.5)
September 1–15	nd	nd	0	0	3.3 (9.1)	4.5 (12.4)	2.9 (6.8)	24.8 (57.4)
September–October	5.3 (4.7)	6.4 (5.7)	3.8 (9.5)	11.5 (28.4)	nd	nd	nd	nd
Summer-spawned bluefish								
August–October	0	0	0	0	1.6 (9.6)	4.9 (28.9)	0	0

Note: %*F*, proportion of bluefish stomachs containing striped bass (±SE); %*W*, proportion contribution of identifiable prey to bluefish diet by weight (±SE). SE, standard error from variance estimators for cluster samples (see equations in the text); nd, no data.

August 5 was 15.4, 13.1, and 3.3 fish·100 m^{-2}, respectively (Table 5). The loss of striped bass during June 30 – July 13, July 13–28, and July 28 – August 10, 1993, was 36.8, 14.0, and 4.4 fish·100 m^{-2}, respectively. Predation mortality estimates ranged from 2.6 to 25.9%·day^{-1} in 1992 and from 2.1 to 5.3%·day^{-1} in 1993 (Table 5). Both the EF/NYDEC and HREMP beach seine survey estimates of striped bass abundance did not decline from late June to early August 1992; during this time period, they either stayed level or increased slightly (Fig. 3). In contrast, there was a dramatic decline in striped bass abundance in late June through mid-July for both surveys in 1993. The EF/NYDEC survey estimates of striped bass abundance continued to decline from mid-July through early August (Fig. 3). The total loss (mortality plus the sum of immigration and emigration) from the 1993 EF/NYDEC striped bass density data from late June to early August was 4.1%·day^{-1}. Bluefish predation in 1993 accounted for 51–129% of total striped bass loss from late June to early August (Table 5).

Relationship between bluefish and striped bass abundance

The NYDEC annual juvenile striped bass index was negatively correlated ($r = -0.61$, $p = 0.022$) with July YOY bluefish abundance (Fig. 4). However, the correlation between the NYDEC annual juvenile striped bass index and the HREMP annual PYSL striped bass index was not significant ($r = 0.08$, $p = 0.746$) (Fig. 4). Hence, striped bass PYSL abundance is not a good predictor of juvenile striped bass abundance, but juvenile striped bass recruitment is significantly related to the abundance of bluefish.

Discussion

Bluefish have long been suspected of causing declines in prey fish abundance (Baird 1873). Here, evidence is presented that bluefish can directly affect the mortality rate of estuarine fish. There were intra- and inter-annual differences in YOY bluefish diets that appear to result from changes in bluefish selectivity as relative abundances of prey change; bluefish avoid striped bass at low densities but select for them at high densities, suggesting a density-dependent feeding response. Striped bass mortality resulting from bluefish predation is at times equal to total juvenile striped bass mortality. Correspondingly, a significant negative correlation exists between striped bass recruitment and bluefish abundance across years. Bluefish predation may account for a significant amount of the previously unexplained density-dependent loss of striped bass between the post-yolk-sac larval and late summer juvenile stages.

Diet and prey type selectivity

Fish prey dominated YOY bluefish diet. This result is similar to past YOY bluefish diet studies in the Hudson River estuary (Texas Instruments Inc. 1976; Juanes et al. 1993; Buckel and Conover 1997) as well as other estuarine and marine systems on the U.S. east coast (see references in Buckel and Conover 1997). Bay anchovy, clupeids (American shad and blueback herring), striped bass, and Atlantic silverside were the most common prey of YOY bluefish in the Hudson River from 1990 to 1993. The dominant prey of Hudson River spring-spawned bluefish from July to September 1974 was bay anchovy, clupeids (American shad and blueback herring), and Atlantic tomcod (Texas Instruments Inc. 1976). These prey accounted for 75% of the diet, while striped bass represented <2%. Juanes et al. (1993) found that bay anchovy, striped bass, clupeids, and Atlantic tomcod dominated the diet of Hudson River spring-spawned bluefish in 1989. Our study confirmed earlier findings that anadromous fish (*Morone* spp. and *Alosa* spp.) often dominate the diet of YOY bluefish in the Hudson River estuary.

Striped bass was a prevalent bluefish prey in 1990, 1992, and 1993 but nearly absent from the diet of bluefish in 1991. This corresponds to low relative abundance of striped bass in 1991 (Fig. 1). Texas Instruments Inc. (1976) found very few striped bass in bluefish diet in 1974, which they attributed to low striped bass abundance. This was in contrast with their preliminary study in 1973 (large striped bass year-class) that found that seven out of the 33 (21%) bluefish examined had striped bass in their stomachs. Other prey types also show interannual variability. Clupeids were more prevalent as prey in 1990 and 1992 compared with 1991 and 1993. Atlantic silverside showed the opposite pattern. It was

Table 4. Prey selectivity (Chesson's α, see text for calculations) in spring-spawned bluefish collected in the Hudson River estuary.

Date	No. of hauls	Striped bass	Clupeids	Atlantic silverside	Other
1990					
July 17–18	8	0.21	0.78	0	0.01
July 31 – August 1–2	12	0.23	0.54	0.04	0.19
August 14–15	6	0.88	0.06	0.06	0
August 28–29	5	0.06	0.33	0.02	0.59
September 11 and October 18	6	0.11	0.23	0.33	0.33
1991					
July 23–24	15	0.12	0.20	0.43	0.25
August 6–7	11	0.11	0.33	0.56	0
August 21–22	5	0	0.24	0.58	0.18
September 3–4	8	0	0.50	0.75	0.13
1992					
June 26 and July 7 and 22–23	14	0.54	0.41	0	0.05
August 5–6 and 18–19	11	0.08	0.75	0.03	0.13
September 1–2 and 15–16	5	0.04	0.74	0.08	0.14
1993					
June 30 and July 13–14	16	0.89	0.07	0.04	0
July 28–29	8	0.53	0	0.39	0.07
August 10–11	6	0.29	0.40	0.32	0
August 24–25	7	0.21	0	0.69	0

Note: Values of $\alpha = 0.25$ represent random feeding, while values of $\alpha > 0.25$ or $\alpha < 0.25$ represent "selection" and "avoidance" of prey, respectively.

a more dominant prey in 1991 and 1993 and less so in 1990 and 1992. Bay anchovy was important in all four years.

Within-year variability in bluefish diet was prevalent in 1990, 1992, and 1993. For example, striped bass was the dominant prey of YOY bluefish immediately after recruitment to the Hudson River estuary in 1992 and 1993. During this time period, striped bass was the primary piscine prey of appropriate size for bluefish. Later in the summer, bluefish began to prey upon bay anchovy, clupeids (1992), and Atlantic silverside (1993) as the relative abundances of these prey increased.

Inter- and intra-annual differences in diet appear to result from changes in the relative abundance of prey. The positive relationship between selectivity and striped bass CPUE suggests that bluefish may exhibit "prey switching" behavior (Murdoch et al. 1975). Switching occurs when the prey that has the highest relative abundance is attacked at a disproportionately higher rate than would be expected from random feeding (Murdoch et al. 1975). The clupeid and Atlantic silverside selectivity data appear to suggest switching behavior as well. For example, in years where clupeids were selected positively (1990 and 1992), the Atlantic silverside was selected against. If bluefish exhibit a type 3 functional response in the presence of multiple prey, then changes in relative abundance of prey that induce switching behavior would result in density-dependent mortality (see Bailey 1994). Other such demonstrations of predator switching behavior are rare (see Townsend et al. 1986).

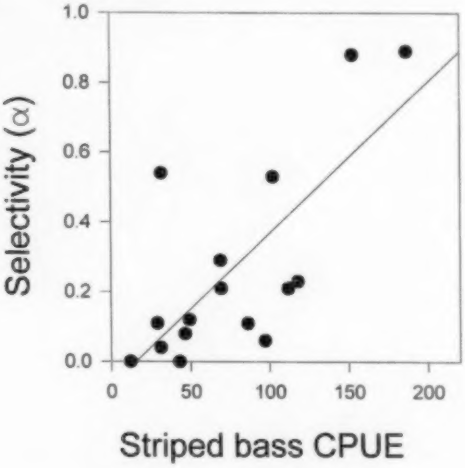
The positive relationship between selectivity and striped bass CPUE could potentially result from covariance between the ratio of striped bass length to bluefish length and the striped bass density. Juvenile striped bass are more vulnerable to bluefish predation at lower prey length to predator length ratios (Scharf et al. 1998). In the Hudson River estuary, the lowest mean striped bass length to bluefish length ratios (measured as mean striped bass length divided by mean bluefish length on a given date) often occur in early summer coincident with higher densities of striped bass. However, the estimates of striped bass selectivity are not significantly related to mean striped bass length to bluefish length ratios ($r = -0.05$, $p = 0.858$).

The amount of overlap that bluefish have with their prey in an estuary will depend partly on bluefish and prey salinity preferences. Bluefish distribution throughout the section of the estuary measured by NYDEC CPUE data does not suggest preference for any area. There are slightly higher CPUE's at the more southern stations, but there was no significant relationship between percent bluefish captured and distance from the mouth of the estuary ($r = -0.16$, $p = 0.067$). Our sampling did not extend as far upriver as bluefish have been captured by HREMP. In years where the salt front is higher and freshwater prey such as clupeids are not found at our beach seine stations, bluefish may still prey on them further upriver where overlap occurs. Therefore, different assemblages seen in the lower Hudson River may result from the salinity level or from real interannual variations in prey fish abundance.

Striped bass loss calculations

Our estimates of striped bass predation mortality show that bluefish predation directly influences prey abundance. For example, our estimates of striped bass density and predation mortality ($25.9\%\cdot\text{day}^{-1}$) suggest that the standing numbers of striped bass would disappear in a relatively short time period. This did not occur in 1992 given that the numbers of striped bass actually increased slightly throughout

Fig. 2. Relationship between bluefish selectivity (Chesson's α) for striped bass and striped bass CPUE in the Hudson River estuary ($r = 0.73$, $p = 0.001$).



the summer. Cohorts that were spawned later in the spawning season or that had initially recruited out of our sampling area may have immigrated into the sampling region. A similar observation was made by Dey (1981) in 1976, a year in which the striped bass spawning season appeared protracted.

However, protracted spawning and late recruitment do not explain how bluefish consumed more striped bass than were present during a given time interval in 1992 (Table 5). This discrepancy could result from either an underestimate of striped density or an overestimate of bluefish consumption, but more likely the former. Striped bass may have occurred in habitats missed by the beach seine in 1992 (e.g., offshore), or peak recruitment in 1992 may have occurred between sampling intervals. Although bluefish consumption estimates are likely not exact (see Potential sources of error section), our estimates show that bluefish consume an amount of striped bass that is greater than or equal to the quantity of striped bass present in early summer. At the very least, this suggests that bluefish are the major source of mortality for juvenile striped bass.

In 1993 the highest density of juvenile striped bass was measured in late June immediately after initial recruitment. Striped bass CPUE declined throughout July and August, allowing estimates of total striped bass loss to be calculated. Our estimate of striped bass loss from June 30 to August 10, 1993, was 4.1%·day^{-1}. This mortality rate estimate is similar to the June 25 – July 26, 1975, Hudson River striped bass late larval and early juvenile mortality rate estimate of 5.1%·day^{-1} (Dey 1981). The three mean bluefish predation mortality estimates during the late June to early August time period explained from half to all of the total striped bass mortality.

Much of the bluefish predation on striped bass occurred during the first few weeks following the appearance of juvenile striped bass in the lower Hudson. From June 30 to July 13, 1993, predation mortality induced by bluefish averaged 5.3%·day^{-1} (± 1 SE, 3.7–6.9) and accounted for 129% (89–168%) of the total striped bass loss from the system. In both 1992 and 1993, over 70% of the total number of striped bass consumed by bluefish throughout the entire summer were consumed by July 14. The declining predation on striped bass after mid-July is due to the lowered incidence of striped bass in bluefish diet. It is not due to declining consumption of prey biomass by bluefish. Total consumption did not decline until August in both 1992 and 1993.

The availability of bay anchovy and Atlantic silverside appears to indirectly affect striped bass survival. Bluefish showed positive selection for striped bass based on the early collections in 1992 and 1993 and then either fed randomly or avoided striped bass during later collections. The low relative abundance of bay anchovy and Atlantic silverside during these early collections likely resulted in higher striped bass predation mortality. For example, the number of striped bass consumed during late July to early August would be 3–3.5 times higher if the proportion of striped bass in bluefish diets stayed at late June to early July levels throughout the summer in both years.

Few studies have examined the influence of estuarine piscivores on the mortality of estuarine fish populations. Wright et al. (1993) measured mortality in spot (*Leiostomus xanthurus*) resulting from southern flounder (*Paralichthys lethostigma*) predation in estuarine ponds. They found that their experimental measurements of spot mortality were similar to field estimates of spot mortality, suggesting that southern flounder predation was significant. Gleason and Bengtson (1996*a*) showed that bluefish feed on inland silverside (*Menidia beryllina*) in Narragansett Bay, R.I., and that this predation was size selective. Larger inland silverside were selected over smaller inland silverside in mesocosm experiments. They went on to examine size-selective mortality in cohorts of inland silverside in the field using back-calculated lengths from otolith–size relationships. Gleason and Bengtson (1996*b*) concluded that size-selective predation throughout the summer months was strong enough to significantly affect the size distribution of inland silverside. They attributed this size-dependent loss to bluefish predation.

Hartman and Brandt (1995) developed bioenergetic models for three estuarine piscivores in Chesapeake Bay: bluefish, weakfish (*Cynoscion regalis*), and striped bass. Although they modeled the cumulative consumption of these fish, the influence of this prey loss on prey populations within Chesapeake Bay was not measured. Their modeling efforts support our findings. Of the three piscivores they examined, bluefish were the most likely to affect prey populations (Hartman and Brandt 1995).

The effect of shrimp predation on recently settled flatfish provides the most conclusive proof of predation affecting the population dynamics of coastal prey populations. van der Veer (1986) showed that variability in abundance of plaice (*Pleuronectes platessa*) declined from the larval to the juvenile stage, indicating that density-dependent mortality reduced variability in year-class strength. Mortality estimates of recently settled plaice can be high (up to 6%·day^{-1}). Shrimp (*Crangon crangon*) predation causes density-dependent mortality in age-0 plaice shortly after settlement, resulting from both the functional and numerical responses

Table 5. Geometric mean striped bass density, striped bass loss resulting from bluefish predation (see the text for calculation description), predation mortality, and percentage of striped bass mortality due to juvenile bluefish predation.

Date	Geometric mean density (no.·100 m^{-2})	Mean estimated predation loss during time period (no.·100 m^{-2}) (±1 SE)	Predation mortality M_{pred} (%·day^{-1}) (±1 SE)	% of total loss (Z + emigration) (±1 SE)
1992				
June 26 – July 7	4.7	15.4 (5.5–25.4)	25.9 (10.1–39.0)	na
July 7–22	6.6	13.1 (5.5–22.4)	13.2 (5.4–20.4)	na
July 22 – August 5	10	3.3 (0.9–6.5)	2.6 (0.6–4.6)	na
1993				
June 30 – July 13	48.2	36.8 (25.2–48.4)	5.3 (3.7–6.9)	128.5 (88.9–167.5)
July 13–28	27.6	14.0 (7.8–20.2)	3.3 (1.9–4.8)	80.6 (45.2–115.6)
July 28 – August 10	14.8	4.4 (1.1–7.8)	2.1 (0.5–3.7)	51.4 (12.9–89.2)

Note: Total loss (mortality plus emigration) was estimated as $Z = 4.1\%\cdot day^{-1}$ for 1993; no estimate of Z could be made in 1992. na, not applicable.

of shrimp to changes in the density of plaice (van der Veer and Bergman 1987).

Potential sources of error

The potential sources of error in our analyses include estimates of relative abundance, consumption rate, and diet of bluefish. Of these potential sources of error, beach seine estimates of relative abundance are the most likely to be biased. Although beach seines have limitations, Pierce et al. (1990) found that capture efficiencies on pelagic fish were relatively high. Additionally, McBride et al. (1995) found that bluefish abundances were underestimated only in beach seines <50–60 m long.

Estimates of striped bass density could have been influenced by seine capture efficiencies during early collections. The numbers of striped bass may have been underestimated due to fish not being retained by the seine mesh. This would lead to underestimates of striped bass total mortality rates and overestimates of bluefish selectivity for striped bass in early collections. Other biases in the calculation of striped bass mortality include fish emigration and immigration. Fish can also escape when the seine hits snags or rolls up on macrophytes (Pierce et al. 1990). The NYDEC beach seine survey monitors these factors during seine sets; the occurrence of these problems was rare in both 1992 and 1993.

The remaining potential sources of error were probably not important in this study. Our field estimates of bluefish daily ration in the Hudson River (Buckel and Conover 1997) are likely robust given their similarity to laboratory (Juanes and Conover 1994, Buckel et al. 1995), bioenergetic modeling (Steinberg 1994), and past field (Juanes and Conover 1994) measurements of bluefish feeding rates. However, point estimates of consumption rate in the field do not account for day-to-day variability in feeding rates. The estimates of bluefish diet are robust given that bluefish were sampled from 25 different beach seining stations covering a large area of the river throughout the summer. This section of the river contains the majority of the juvenile striped bass population (see Materials and methods).

Very few other studies of predation mortality have estimated the variability around predation mortality estimates. Using primarily the delta method (Seber 1973), standard error estimates of bluefish density, weight, daily ration, diet, and prey sizes were used to calculate an estimate of error around the mean numbers of striped bass consumed by bluefish; for example, the mean (± SE) loss of striped bass to bluefish predation was 36.8 (± 11.6) fish·100 m^{-2} from June 30 to July 13, 1993 (Table 5). Given that confidence limits are roughly equal to ± 2 SE of the mean (Sokal and Rohlf 1981), the confidence limits for this estimate would range from 13.6 to 60 fish·100 m^{-2}. These large confidence limits point out the uncertainty of our estimate and the difficulty in quantifying predatory impacts in large systems. Field sampling should be designed to aid in reducing variability around estimates of diet, daily ration, predator and prey density, and other required data.

Bluefish predation explained a large amount of striped bass mortality in a year when bluefish density was relatively low. Therefore, the ability of bluefish to affect striped bass or other estuarine prey populations at high bluefish density years may be larger than estimated in this study in 1993.

Relationship between bluefish and striped bass abundance

The NYDEC juvenile striped bass abundance index and HREMP's PYSL index for striped bass were uncorrelated. Years with a large PYSL abundance do not correspond to a large juvenile striped bass year-class. Pace et al. (1993) also examined the relationship between PYSL abundance and juvenile striped bass abundance but used HREMP data for both PYSL and juvenile striped bass. They also found no relationship between these factors and concluded that either (*i*) recruitment to the juvenile stage is not dependent on larval abundance or (*ii*) the juvenile striped bass abundance estimates are in error.

Bluefish abundance explained a significant amount of the variation ($r = -0.61$, $p = 0.022$) in juvenile striped bass recruitment. Therefore, bluefish predation may account, in part, for the lack of a relationship between PYSL abundance and recruitment of juvenile striped bass in the lower Hudson River. Further studies into the mortality at metamorphosis and the early juvenile stage are needed to better understand the early life history of the Hudson River striped bass stock.

Management implications

Since the early 1960's, there has been a large effort to assess the impact of Hudson River powerplant entrainment on the abundance of the early life history stages of striped bass

Fig. 3. Estimated Hudson River striped bass density ± 1 SE (circles, from EF/NYDEC beach seining) and cumulative loss to bluefish predation (solid line) for (A) 1992 and (B) 1993. Broken lines represent the cumulative consumption of ± 1 SE of the daily numbers of striped bass consumed by bluefish per 100 m^{-2}. CPUE of striped bass from HREMP beach seine sampling is also shown (squares).

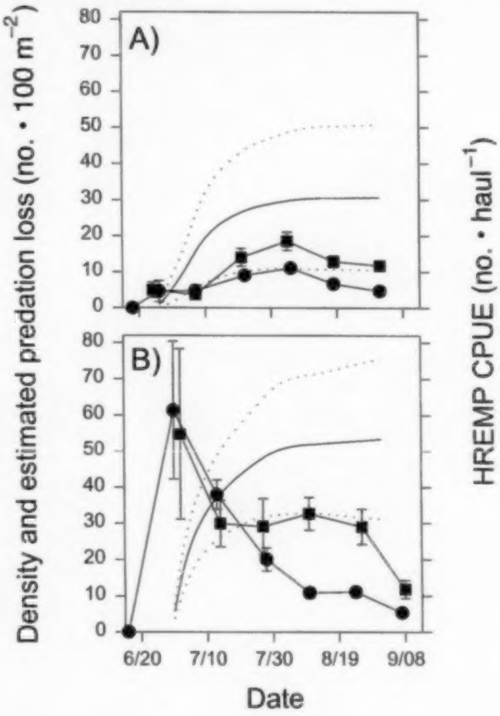
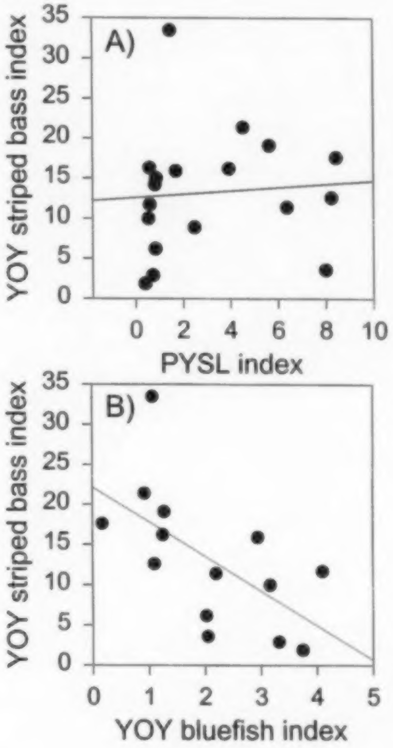


Fig. 4. Annual geometric mean Hudson River juvenile striped bass index from the NYDEC seine survey as a function of (A) the HREMP PYSL index ($r = 0.08$, $p = 0.746$) and (B) the annual bluefish index ($r = -0.61$, $p = 0.022$) developed from the mean CPUE of YOY bluefish from HREMP sampling during the month of July.



(Barnthouse et al. 1988). It is still unknown if powerplant entrainment affects striped bass population dynamics. However, it is known that the entrainment mortality of the early life stages of bay anchovy is exceedingly high (35–79% of the standing stock; Boreman and Goodyear 1988). Powerplant entrainment of bluefish forage species, such as bay anchovy, may indirectly affect striped bass mortality. Bluefish preference for striped bass prey increased as a function of striped bass relative abundance. Hence, a reduction in bay anchovy abundance due to powerplant entrainment may lead to an increase in bluefish predation on striped bass. Management of powerplant entrainment should not be concerned only with direct entrainment of striped bass (see Barnthouse et al. 1988) but also with the consequences of the removal of bluefish forage (e.g., bay anchovy early life history stages) on striped bass survival.

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Behavioral inferences from regulatory observer data: catch rate variation in the Scotian Shelf silver hake (*Merluccius bilinearis*) fishery

D.M. Gillis

Abstract: The detailed spatial and temporal data collected for the regulation of the silver hake (*Merluccius bilinearis*) fishery on the Scotian Shelf provide a unique opportunity to test hypotheses about variability in catch rates on the scale of individual trawls. I used these data to examine vessel interactions and long-term temporal trends in catchability. An index of course linearity, derived from observed positions, times, and speeds of fishing vessels, indicated that interference competition was present in the fishery. However, catch rate did not decline with local vessel density. This apparent contradiction is consistent with fleet dynamic theory and suggests that the direct examination of catch rates is a poor test for interference in the retrospective analysis of fisheries data. The study of extended periods of high, localized fishing activity revealed a cycle in catch rates with periods of about 6 days. Such periods may represent an interaction between tidal, diel, and (or) technological factors. Although more study is required to identify the cause of these cycles, their existence should be considered in the design of surveys and other population studies using catch and effort data.

Résumé : Les données spatiales et temporelles détaillées recueillies pour la réglementation de la pêche du merlu argenté (*Merluccius bilinearis*) sur la plate-forme néo-écossaise offre une occasion unique de tester les hypothèses concernant la variabilité des taux de capture d'un chalut à l'autre. J'ai utilisé ces données pour examiner les interactions entre les bateaux et les tendances temporelles à long terme de la capturabilité. Un indice de la linéarité des routes, obtenu à partir des positions, heures et vitesses observées des bateaux de pêche, a indiqué qu'il y avait compétition par interférences dans cette pêche. Cependant, le taux de capture ne diminuait pas avec la densité locale des bateaux. Cette contradiction apparente va dans le sens de la théorie de la dynamique des flottilles et laisse penser que l'examen direct des taux de capture évalue mal l'interférence dans l'analyse rétrospective des données de pêche. L'étude de périodes étendues d'activité de pêche intense et localisée a révélé que les taux de capture suivent un cycle dont la période est d'environ 6 jours. Des périodes de cet ordre peuvent être fonction d'une interaction entre des facteurs maréaux, nycthéméraux et (ou) technologiques. Bien qu'on doive pousser les recherches pour identifier la cause de ces cycles, leur existence devrait être prise en considération dans la conception des campagnes d'observation et d'autres études des populations utilisant des données de capture et d'effort de pêche.

[Traduit par la Rédaction]

Introduction

The fundamental assumption underlying the use of catch per unit effort (CPUE) as an index of abundance is that catch rate is proportional to overall abundance. However, the potential for biases in this relationship is well established in the fisheries literature (e.g., Paloheimo and Dickie 1964; Clark and Mangel 1979; Cooke 1985; Mangel and Beder 1985; Swain and Sinclair 1994; Hutchings 1996). Previously (Gillis et al. 1993; Gillis and Peterman 1998), my coauthors and I suggested that vessel movement in response to catch rates may also break down the relationship between CPUE and abundance, especially if those movements follow the ideal free distribution (IFD) (Fretwell and Lucas 1970; Fretwell 1972). Recent work indicates that IFD's can be directly applied to the interpretation of catch and effort data from a variety of fisheries including salmon, groundfish, and whales (Abrahams and Healey 1990; Whitehead and Hope 1991; Healey and Morris 1992; Gillis et al. 1993). In addition the IFD can also provide the basis for alternative indices of abundance (Gillis and Peterman 1998). However, the formation of an IFD that alters the relationship between local abundance and catch rate requires the presence of interference competition among fishing vessels.

Interference refers to any rapidly reversible reduction in foraging efficiency that is associated with an increase in foraging activity (after Goss-Custard 1980). In the case of fishing, interference could include vessel interactions such gear entanglement, a reduction in efficiency due to the avoidance of gear entanglement, or the temporary dispersal of fish aggregations in response to fishing. Although interference seems likely in situations with large fleets or concentrated fishing activities, it has not yet been quantitatively demonstrated during commercial fishing.

Another potential source for disruption of the relationship between CPUE and abundance is a systematic variation in catchability throughout the period when data are collected. Catchability may vary independently of stock size as a result

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of the natural movements of the target species. In the case of silver hake (*Merluccius bilinearis*) on Georges Bank, it has been shown that the catch rates obtained by trawling with roller gear are better at night when trawling targets fish leaving the bottom to feed (Bowman and Bowman 1980); however, changing gear can reverse this trend. Also, the influence of tidal currents on the availability of fish or the efficiency of gear deployment is a potential source of variation in catchability that is poorly studied.

Silver hake from the Scotian Shelf has traditionally been a valuable source of animal protein and export revenue in both Russia and Cuba. These fish are concentrated along the shelf break at depths of about 200 m, but they migrate onto shallower regions of the shelf in the late summer to spawn. Concerns about regulatory compliance and the bycatch of other commercially valuable species such as haddock (*Melanogrammus aeglefinus*) and Atlantic cod (*Gadus morhua*) led to the implementation of the International Observer Program (IOP) by the Canadian government in 1977.

The IOP places contracted observers on foreign vessels fishing within Canadian waters. These individuals collect detailed information about the contents and disposition of each trawl. Their data is then compiled and stored at the Bedford Institute of Oceanography, Dartmouth, N.S., where they are used to examine current management issues.

My goal in this paper is to provide an example of how regulatory data can be used to investigate aspects of fish and vessel behavior that are relevant to the interpretation of catch and effort statistics but difficult to study directly. Specifically, I will use the regulatory observer data to (*i*) quantify the spatial scale on which fishing vessels interact and (*ii*) examine time trends in catchability within the silver hake fishery.

Materials and methods

Data set

The information used in these analyses was taken from the records of the IOP for the 1993 foreign fishery targeting silver hake. This fishery was prosecuted by 24 vessels (12 Cuban and 12 Russian) using bottom-trawling gear. These vessels were of similar dimensions, varying between 2300 and 3300 gross registered tons in capacity and between 80 and 110 m in length. The season for this fishery ran from April 1 to November 15, although some exploratory fishing was allowed in late March. The fishery was restricted to a region known as the "Silver Hake Box" in order to reduce interference with domestic fisheries (Fig. 1*a*).

The observation of foreign fishing was conducted by independent, contracted observers with about 100% coverage of all foreign fishing activity within Canadian waters. The records of these observers included information about catch composition, gear configuration, the initial and final positions, and the initial and final times of each trawl. These data were then transferred to electronic form and made available through the observer database, which is maintained under the supervision of M. Showell (IOP, Bedford Institute of Oceanography, Dartmouth, NS B2W 4A2, Canada).

Vessel interactions

No quantitative information on vessel interactions was provided in the original data set. In order to perform tests on vessel behavior, I used the records of trawl location and time to generate estimates of the nature of the course followed (trawl linearity) and the local density of other fishing vessels. These proxies for fine-scaled temporal and spatial fleet dynamic data were then used in subsequent tests of hypotheses examining the potential influence of vessel density on the course and success of a trawl.

Linearity of trawl

Under crowded fishing conditions, vessels may need to alter course during a trawl to avoid gear entanglement or collision. Thus, extreme deviations from linear courses may indicate the presence of vessel interactions. A deviation from a linear course would result in the distance covered by a vessel exceeding that indicated by its change in geographical position. However, due to variation in trawl durations, both temporal and spatial displacements had to be considered in the analysis. I developed an index of trawl linearity based upon observed and apparent speed by subtracting the speed recorded by the observer of the vessel (speed through water) from the speed estimated by the vessel's change in latitude and longitude during the trawl. For convenience, this was called the speed index (SI). When trawls followed a straight line, the value of SI would be zero. If vessels purposefully altered their course around a vessel or another obstacle, the SI would be negatively biased due to the nonlinear course followed. However, currents should also affect the SI. If trawls occurred both directly into and with a prevailing current and without vessel interactions, the SI would be expected to show deviations around a mean of zero due to the drift that occurred while fishing. Any constant course where a portion of the vessel's velocity was contrary to a prevailing current would negatively bias SI. Conversely, any component of the vessel's velocity that was in the same direction as the prevailing current would positively bias the SI. Occasionally, vessels reversed their courses without removing their nets from the water in order to fish continuously in the same location. This was recorded as a "turn-around set" by the observers. Such behavior invalidated the use of SI as a potential indicator of vessel interference, so these trawls were not included in the quantitative analyses. The distribution of SI values was examined by plotting their histogram and calculating the skewness of their distribution (excluding turn-around sets).

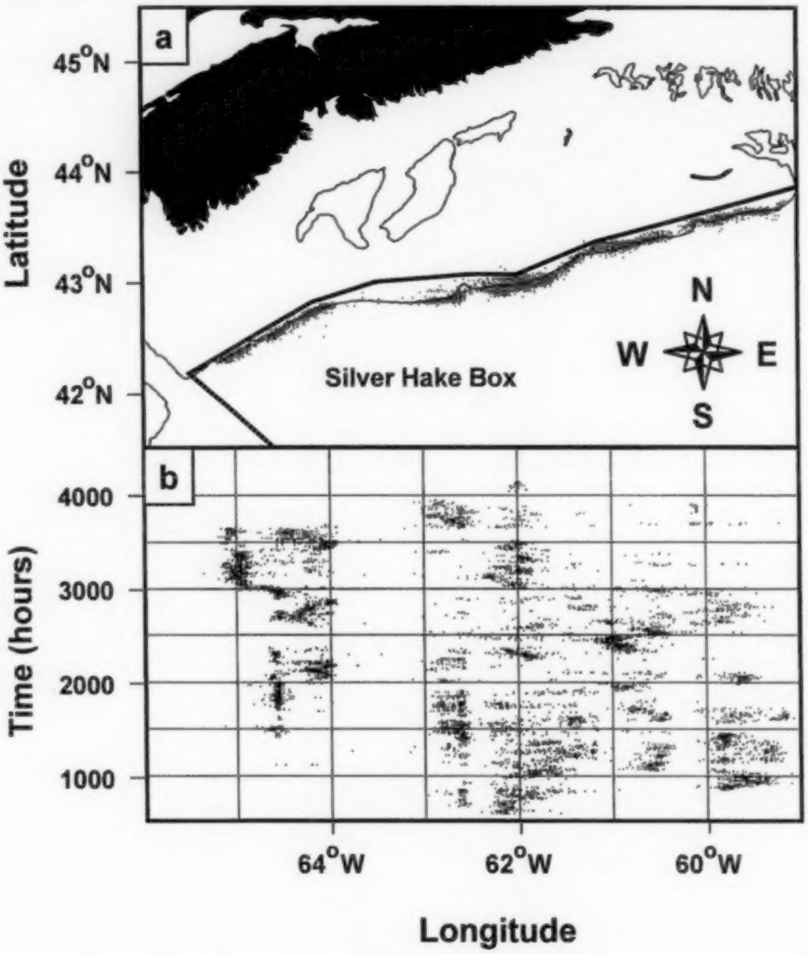
Estimate of vessel density

An estimate of local vessel density was required in order to make inferences about the spatial scale of vessel interactions. Initial and final positions had been recorded to 1′ of latitude and longitude (about 1 nmi (1852 m)). Using each trawl in turn as the focus, I reconstructed the position of all actively fishing vessels, beginning at the start of the focus trawl and recalculating the position of all vessels that were actively fishing every 5 min until the end of the focus trawl. This was done by assuming that all trawls followed linear courses (but see above) and interpolating their positions at each time interval. These reconstructed positions were then used to estimate the number of vessels within specific ranges (0–1, 1–2,..., 9–10 nmi) of the focus trawl. The maximum number of vessels occurring within each range was estimated as the highest count of vessels within that range during the focus trawl. Clearly, these density estimates will be more inaccurate when trawls are less linear in nature. However, this reconstruction provides a useful indictor of potential interactions and an index of vessel crowding to explore the distance over which these interactions may occur.

Inference of vessel interactions

A multiple linear regression of SI on the maximum number of vessels estimated for each of the ranges was used to examine the hypothesis that vessel density affected the courses followed during a trawl. The underlying assumption in this analysis was that a negative bias in SI would result from a nonlinear course being followed to avoid gear entanglement or other detrimental effects of fishing in close proximity to other vessels. The potential multicollinearity problems in the analysis were dealt with by examining

Fig. 1. Temporal and spatial distribution of fishing effort in observed trawls of the Russian and Cuban silver hake fleets. (*a*) The bathymetry is represented by the 200-m contour. The Silver Hake Box is the region where the fishery was permitted by Canadian authorities. (*b*) Time is measured as hours after 00:00 GMT on May 1, 1993. In both Figs. 1*a* and 1*b*, the dots represent the mean position of a trawl; 9880 trawls in total are represented. The contours and positions in Fig. 1*a* are plotted as simple geographical coordinates, so the same longitude scale applies to both parts of the figure.



the variance inflation factor (VIF) associated with each predictor variable. The VIF has an ideal value of 1, with serious multicollinearity problems being indicated by VIF values in the range of 10 or more (Neter et al. 1990).

Periodicity in catch rates

All catch rates were initially calculated as the average CPUE of a single trawl by dividing the catch in that trawl by the duration of the trawl. These CPUE values were then transformed by common logarithms to reduce heterogeneity among variances and deviations from normality. All parametric tests were performed on the transformed values, while nonparametric tests used the untransformed CPUE values.

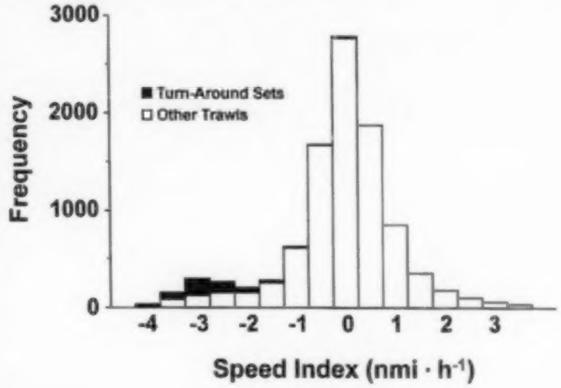
Daily variation in catch rates

Potential diel patterns in catch rates were examined by plotting the catch rate (as $\log_{10}$ CPUE) on the time of the start of each trawl (in Greenwich mean time (GMT)). The diel trend was tested by comparing catch rates between trawls started in daylight hours and during the night using a *t* test. The proportion of the variation in $\log_{10}$ CPUE explained by a difference between the day and night means was estimated as the r^2 from the nonlinear regression of this two-state model. This and all subsequent nonlinear regressions were performed using the CNLR program from SPSS (SPSS Inc. 1997).

Longer cycles

The potential for cycles in catch rates with periods in excess of 24 h was examined by choosing periods of high, localized fishing activity that extended for greater than 1 week in duration. A simple clustering algorithm was used to identify these fishing "events" based upon the proximity of trawls in space and time relative to the entire data set. Trawls were grouped together if their distance was less than the 50th percentile of distance traveled during a trawl (7.1 nmi). Differences between the starting time of trawls were converted to distances by multiplying by the 10th percentile of the calculated trawling speeds (1.9 kn (1 kn = 0.51 $m \cdot s^{-1}$)). The Euclidean distance between trawls was then calculated in both spatial and temporal dimensions. A new trawl was joined to a group of

Fig. 2. Distribution of speed index values in the silver hake fishery. The black portions of the bars represent the 485 turn-around sets where the vessels reversed their course without retrieving their nets in order to fish the same area twice. Open bars represent the remaining 9258 trawls.



trawls if it was within the distance criterion for any other trawl in the group. This methodology allowed for the formation of irregularly shaped aggregations of trawls that could potentially track the movements of fish through space. The final groupings form aggregations of proximate trawls that are useful for focused analysis, but these aggregations are not meant to represent definitive patches of fishing activities.

Groupings of trawls that persisted for longer than 1 week were the focus of subsequent analysis. The examination of diel patterns in catch described above and previous work on silver hake (Bowman and Bowman 1980) suggested that catch rates should vary regularly over a 24-h period. Therefore, $\log_{10}$ CPUE values of trawls were averaged for each day. As it seemed probable that such focused fishing activity would deplete or disperse aggregations of fish, the $\log_{10}$ CPUE values were detrended by regressing the averages on time. This linear model, fitted to the $\log_{10}$-transformed CPUE values, implied that catch rates declined in the exponential manner expected from the classical Baranov catch equation (Ricker 1975). The residuals of this regression were used to test for periodicity by fitting them to the wave equation

$$\log_{10} \mathrm{CPUE} = \mathrm{Amplitude} \cdot \cos\left(2\pi \frac{(\mathrm{Day} - \mathrm{phase})}{\mathrm{Period}}\right)$$

by nonlinear regression. The significance of this relationship was tested by randomization of the residuals. In each randomization, the residuals were randomly reordered with respect to time, creating a new data series. The wave equation was then fitted to the "shuffled" data. The significance of the original r^2 values was estimated by calculating the proportion of randomized analyses that produced r^2 values greater than or equal to the observed value. One thousand randomizations were initially performed. Where the significance was estimated to be less than 0.10, an additional 4000 randomizations were performed. The period of the cycle was represented by the 95% confidence interval constructed from 1000 bootstrapped samples of the original data.

Details of event A

The trawls of the longest event were examined in greater detail in order to assess the relative impact of event duration, periodicity, and vessel variations on catch rates. This event was chosen as being the sample of fishing activity most likely to provide insights into possible vessel interactions and temporal trends. The amount of variation in the $\log_{10}$ CPUE values explained by the different factors was estimated by the r^2 values of the associated linear and nonlinear regressions. The proportion of the variance explained by vessel interactions could not be estimated due to the nature of the results. Finally, the relative performance of vessels with more than 30 trawls occurring in event A was compared by nonparametric ANOVA of CPUE values. A parametric ANOVA was inappropriate due to the heterogeneity among the variances of the transformed CPUE values for the individual vessels.

Results

Descriptive summary of fishing activity

The characteristics of a typical silver hake trawl were obtained by examining subsamples of the 9880 observed trawls for which the necessary information was on record. Trawls averaged 2.24 h in duration (SD = 0.82, n = 9855) at a mean logged speed of 3.4 kn (SD = 1.13, n = 9762). The course, calculated from the position at the beginning and the end of the trawl, was within one compass point (22.5°) of either east–northeast or west–southwest in 9134 of 9785 trawls. The mean depth trawled was 211 m (SD = 65.5, n = 9880). The observed catch of a single trawl ranged from 0.25 to 24 000 kg of silver hake, with a mean of 1413.4 kg (SD = 1525.1, n = 9855).

The geographical distribution of the 9785 trawls with complete positional data on record was concentrated along the seaward edge of the Scotian Shelf, approximately following the 200-m depth contour (Fig. 1*a*). The nearly linear distribution of effort along the top of the continental slope (or "shelf break") allows the temporal trends in spatial distribution to be summarized by a plot of longitude through time (Fig. 1*b*). Throughout the 1993 fishing season, trawls occurred at varying levels of aggregation. At some times (such as from 1500 to 2000 h, between 64 and 65°W longitude on Fig. 1*b*), fishing effort appears tightly aggregated; however, at other times (such as from 500 to 2000 h, around 62°W longitude on Fig. 1*b*), fishing activity occurs diffusely within a definable area. Periods of fleet movement while fishing (such as around 3000 h from 64.5 to 65°W) appear as nearly horizontal and diagonal collections of points in Fig. 1*b*. Nearly vertical assemblages of points represent effort that is concentrated within a limited region over time. There is no apparent dichotomous distinction between "patches" of activity and the surrounding areas. Instead, there appears to be a continuum of aggregation in the distribution of effort, ranging from isolated events to tight clusters.

Vessel interactions

Linearity of trawls

The distribution of the SI, excluding turn-around sets, ranged from –4.5 to 3.75 around a single mode (Fig. 2). The distribution had a mean near zero (mean = –0.007) and an SD of 1.02 (n = 9258) among all trawls (excluding turn-around sets). This pattern would be expected if much of the deviation in SI resulted from drift in currents running parallel to the courses trawled. The turn-around sets provided a reference point for known nonlinear trawls and indicated that they do produce negative SI values (Fig. 2). The distribution of SI values was negatively skewed, even when turn-around sets were excluded from the analysis (skewness = 0.529, SE

Table 1. Summary of the range–ring regression.

Range	Slope	SE_{slope}	t	p	VIF
0–1	–0.13	0.03	–4.48	<0.0001	1.59
1–2	–0.11	0.02	–4.49	<0.0001	2.25
2–3	–0.06	0.02	–3.18	0.0015	1.74
5–6	–0.06	0.02	–3.43	0.0006	1.47
7–8	–0.08	0.02	–3.85	<0.0001	1.70
9–10	0.04	0.02	2.33	0.0197	1.48

Note: Observations from turn-around sets were excluded from the analysis. Slopes, their SE's (SE_{slope}), t values for the null hypothesis of slope = 0 (t), significance of the t value (p), and variance inflation factor of the coefficient (VIF) are presented for ranges that had a statistically significant effect on the speed index. The r^2 for the regression was 0.03 ($F_{6,8760} = 45.83$, $p < 0.0001$).

= 0.025, n = 9258). This lack of symmetry is consistent with the presence of trawls that have departed from linear courses by varying degrees. The small but significant positive correlation between CPUE and SI (Spearman's r = 0.018, p = 0.044, n = 9258) was consistent with the association of SI values with poorer fishing success.

Inference of vessel interactions

The index of trawl linearity declined with increasing local vessel density. The stepwise regression of SI on vessel densities at different ranges (Table 1) identified significant effects at ranges of up to 10 nmi However, the magnitude of the slopes showed that the strongest effects occurred within the first 2 nmi, and the direction of the relationship even reversed at 10 nmi. The negative slopes (Table 1) indicated that increased vessel densities were associated with reduced SI values and therefore a tendency for less linear courses. Multicollinearity among the predictor variables was not a concern in this case because none of the VIF's for the predictor variables were greater than 3.

The profitability of trawls was slightly greater when local vessel density was higher. The regression of $\log_{10}$ CPUE on the maximum number of vessels within 2 nmi of an actively fishing vessel revealed a slight, positive relationship (slope = 0.07, $F_{1,9707} = 95.3$, $p < 0.0001$) over the entire data set.

Periodicity in catch rates

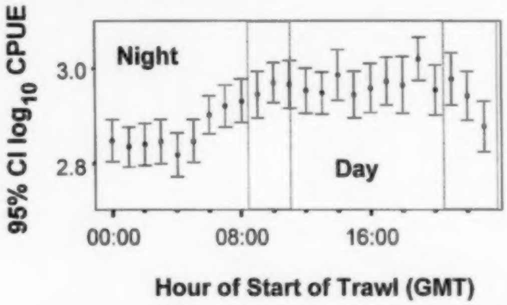
Daily variation in catch rates

A general diel pattern in catch rates was observed, with catch rates increasing towards sunrise and remaining higher throughout the day (Fig. 3). A comparison between day and night trawls (excluding trawls that started within the range of daybreak and nightfall) confirmed this observation (t = –8.80, df = 7511, $p < 0.001$). However, the difference between the $\log_{10}$ CPUE means for day and night trawls only accounted for about 1% of the total variation, as estimated from the nonlinear regression of the day–night model.

Longer cycles

The examination of concentrations of fishing activity indicated that additional periodicity, beyond diel effects, was present in the data. The clustering of trawls that occurred in close proximity to each other defined four events that were 10 days or longer in duration. These events were located throughout the area fished, focusing near 65.0°W (event A, 2880–3402 h), 61.0°W (event B, 2325–2609 h), and 64.3°W (event C, 2661–2895 h, and event D, 2040–2243 h) along the shelf break. For the event with the longest duration (event A), average initial catch rates appeared to increase through time rather than decline. These initial trawls also corresponded to a period of westward fleet movement along the shelf break (Fig. 1*b*, 2880–3048 h, around 65°W), which may have represented an exploratory phase in fleet activities. The initial 7 days of event A were truncated to allow the linear detrending of the remaining data. In the remaining events, there was no visible evidence of fleet movements, and catch rates were successfully detrended by a linear model without censoring the data. The examination of the detrended daily mean of the $\log_{10}$ CPUE values suggested a cycle with a period that was somewhat less than 1 week in duration (Fig. 4; Table 2). However, the fit of the data to the cyclic model, as defined by r^2, declined rapidly with the duration of the event and was only significant for event A. This illustrates the need for prolonged local fishing activity to generate data that can detect such trends.

Fig. 3. Diel pattern in silver hake catch rates. The 95% confidence intervals for the hourly averages of $\log_{10}$ CPUE are displayed based upon the 9880 trawls observed in the 1993 season. CPUE was originally measured in $kg \cdot h^{-1}$. The narrow bands between the dotted lines represent the ranges of daybreak and nightfall throughout the fishing season. The hour of the averaged trawls is given as GMT.

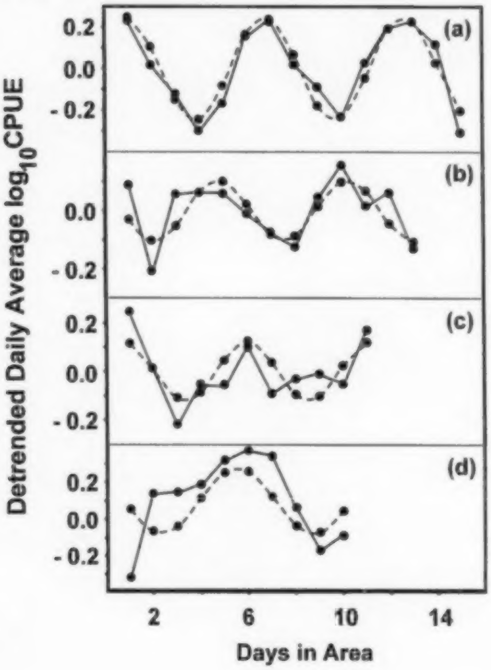


Details of event A

The further analysis of the variance in $\log_{10}$ CPUE within those trawls that were clustered into event A emphasized the level of randomness of catch rates, even during periods of concentrated, localized fishing activity. Only 20% of the observed variance could be accounted for by variation in the daily means (linear regression, df = 1, 614, $p < 0.001$). The linear decline in daily means accounted for about 15% of their variance, but this trend was not statistically significant (linear regression, df = 1, 13, p = 0.0826). However, due to the near significance of this regression, the detrended time series were used in subsequent analysis. A nonlinear fit of the wave equation determined that nearly 90% of the variation in the detrended daily means (about 15% of the total variance in $\log_{10}$ CPUE) could be associated with the long period cycles (Table 2).

The residuals of the cyclical model were found to differ among vessels but not between day and night trawls. A two-way ANOVA of these residuals had to be abandoned due to heterogeneity in the variances. Kruskal–Wallis one-way

Fig. 4. Periodic trends in local catch rates. The absissa represents the amount of time during which a portion of the fleet has been aggregated in a single area. The broken curve is the fitted cycle and the solid line represents the observed data. CPUE was originally measured in kg·h^{-1}.



ANOVA revealed a vessel effect on catch rates (df = 8, p = 0.0032). Although they differed, a plot of vessels' catch rate means and SD's (Fig. 5) illustrates that the vessel effect is small and the variability of catch rates among vessels is heterogeneous. A Mann–Whitney U test was unable to confirm the difference between the catch rates of day and night trawls that was previously observed in the overall data set (n_1 = 237, n_2 = 233, p = 0.2656).

The catch rates within event A were similar regardless of how closely the vessels fished. An ANOVA comparing $\log_{10}$ CPUE found no significant difference among trawls with zero, one, or two other trawls approaching within 2 nmi of their activity (df = 2, 613, p = 0.405).

Discussion

This analysis of the 1993 silver hake fishery illustrates that regulatory data can also be used to examine details of fleet dynamics that are relevant to the interpretation of commercial data and research survey design. The lack of formal experimentation will always limit the conclusions that can be drawn from such an analysis. However, this limitation is offset by both the quantity of data potentially available and the collection of data during the normal prosecution of the fishery. In the case of the IOP data examined here, three key points have emerged: (*i*) interference effects may not result in variation in local catch rates during normal fishing operations, (*ii*) vessel movement data may be better suited to the detection of interference than catch rates alone, and (*iii*) the examination of data from commercial fisheries can reveal trends that are unlikely to be observed in typical research surveys.

Table 2. Summary of the fitting of the wave equation to logged and detrended CPUE data.

Event	Mean no. of vessels	95% CI of period (days)	r^2	p value of r^2	Duration (days)
A	8	5.55–6.17	0.9	<0.01	15
B	9.92	4.45–6.43	0.54	0.07	12
C	5.8	3.49–6.86	0.52	0.14	11
D	7.2	5.54–7.25	0.32	0.43	10

Note: The mean number of vessels refers to the average of the number of vessels participating in an event on the same day. The confidence interval (CI) of the period is estimated from 1000 bootstrapped samples of the original data. The significance of the relationship was estimated by repeatedly fitting the wave equation to randomized data sets created from the original logged and detrended CPUE data and enumerating the proportion of cases where the r^2 value was equal to or greater than the observed value. One thousand randomizations were performed initially and 5000 randomizations were completed for those cases where p was estimated to be less than 0.10.

Previous attempts to test for interference among fishing vessels are rare, and they have met with limited success. Abrahams and Healey (1993) found that troller catch rates declined for chinook salmon (*Oncorhynchus tshawytscha*) and increased for spiny dogfish (*Squalus acanthias*) when vessels fished experimentally in closer proximity. Vignaux (1996) found no significant relationship between increasing vessel densities and catch rates in the New Zealand hoki (*Macruronus novaezelandiae*) fishery. In my analyses, CPUE's over the entire fishery tended to be slightly higher when there were greater numbers of vessels fishing within 2 nmi of a trawl. On a smaller scale (event A), catch rates seemed unrelated to local vessel densities.

In the presence of interference, poor or even positive correlations between catch rates and vessel densities are to be expected in unrestricted fishing situations. This is analogous to Hilborn and Ledbetter's (1979) discussion of the effect of costs on CPUE's in neighboring areas. Briefly summarized, if an area is more costly (in terms of travel or risk), then the mean CPUE required before vessels fish there should be greater than alternative areas. This will result in catch rates that appear "better" in more costly areas and similar in areas with similar costs (but not necessarily abundances). Alternatively, these higher catch rates could also be the result of information exchange and the ability of the fleet to locate and track fish aggregations. Only in an entirely random fishery, or within the restrictions of a controlled experiment, would catch rates be expected to appear depressed when vessel densities are high.

Within a local area, such as event A, vessels may space themselves to minimize the disturbance to the aggregations of fish being caught. Cod, herring, and capelin have all been observed to respond to the sound of vessels (see Mitson 1995 for a review), even in the absence of fishing activities. Where interference is the product of vessel avoidance, vessels should fish more closely only when high fish densities compensate for the reduction in catch rate resulting from the

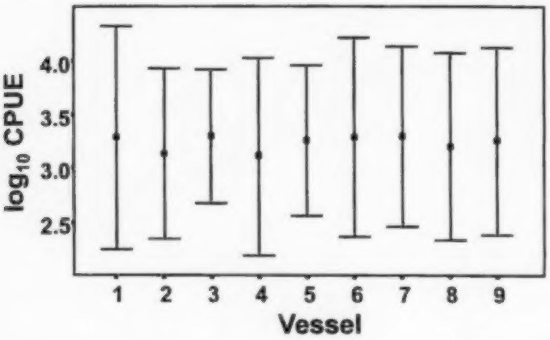


Fig. 5. Mean and SD (×2) of $\log_{10}$ CPUE for all vessels participating in event A (see Fig. 4*a*) that performed more than 30 trawls. There was an overall difference in catch rates among the vessels (Kruskal–Wallis one-way ANOVA, df = 8, p = 0.0032), but this figure illustrates that the variability in catch rates was high and the actual differences between means were small.

avoidance of vessel and gear noise. Thus, average catch rates could be equal, even when the fish are distributed unevenly throughout the area being exploited. In general, the relationship between costs and benefits that appears to influence effort distribution in many fisheries (Hilborn and Ledbetter 1985; Abrahams and Healey 1990; Hilborn and Kennedy 1992; Gillis et al. 1993) implies that the examination of patterns in vessel movements is critical to identifying the role of interference in a fishery.

The SI developed here provides a useful tool for inferring the details of vessel movements from data of limited spatial resolution. However, the patterns in SI will be easier to interpret when fishing occurs parallel to the prevalent currents. On the Scotian Shelf, the summertime current at the surface (<30 m) along the shelf break generally runs parallel to it (Gregory and Bussard 1996). Thus, trawls tended to occur either into or with the current, resulting in the unimodal distribution of SI's (excluding turn-around sets) observed in Fig. 2. In the case of a fishery that trawled perpendicular to the prevalent current, the mean SI would tend to be negative due to the component of the course steered by a vessel that compensated for drift, but the shape of the distribution of SI's could still provide evidence of nonlinear trawls. Finally, nonlinear trawls could be generated from a fleet that closely followed meandering depth contours during fishing. The SI distribution from such a fishery would have negative mean SI. In these silver hake data, large, negative SI values occurred throughout the entire area fished and adjacent to other hauls with less extreme SI values, so it seems unlikely that bathymetry explains the pattern of SI values.

The inference of interference from nonlinear trawls is not unequivocal. Here, I have shown that over the whole fishery, less linear trawls have lower catch rates, but within a localized area and time (event A), there is no relationship between SI and catch rate. This latter observation is to be expected for the reasons previously discussed. I have presented the former as evidence that nonlinear trawls are less profitable in general, but this relationship is weak and could easily be the result of an unquantified covariant.

The clearest way to document interference would be to perform an experimental study in which vessel densities are controlled within a local area. However, such experiments are costly and even potentially dangerous due to the risks of gear damage and injury. In addition, the results may not be clear; the catch rates of different species may respond uniquely to vessel density, as was the case in Abrahams and Healey (1993). When such experiments are conducted, it will be important to chose experimental conditions (fish distributions, vessel densities, vessel movement patterns, and the criteria for the evaluation of profit) that are within the scope of commercial fishing activities in order to extrapolate the experimental results to the fishery. Indirect indicators such as SI can be useful for identifying situations where the evidence of interference is strong enough to warrant further study.

The range–ring regressions of the SI provide a first approximation of the spatial scale over which vessel interactions are occurring. The reconstruction of vessel distributions used to perform this analysis is based upon many assumptions (see Materials and methods), and therefore, the pattern of the results for different ranges should be considered in the interpretation of results, rather than the significance of any single regression coefficient. In this case, the lower magnitude of the slopes at ranges beyond 3 nmi suggests that the strongest interactions occur at 2 nmi or less. This is the range that any future field experiment on interference in the silver hake fishery should focus upon.

The presence of diel and long period variability in catch rates throughout the fishery should be explicitly considered in the interpretation of catch and effort data and the design of research surveys. It is unlikely that a survey of a few weeks in duration will disclose the presence of long period cycles because of the spatial variability in catch rates experienced due to other factors. To isolate these cycles, a directed study is required. Bowman and Bowman (1980) trawled every 3 h for 2 days from a single vessel in order to produce data indicating the presence of a diel cycle for silver hake on Georges Bank. A similar sampling protocol testing for weekly cycles would be costly; furthermore, it may be difficult to locate a single concentration of fish that persists over the entire study. However, such data can potentially be extracted from commercial fisheries when detailed observer records are available. If long period cycles in catch rates are discovered, their potential impact on the efficiency of survey designs can be readily evaluated.

The mechanism for cycles in the catch rates of silver hake on the Scotian Shelf cannot be established from the commercial fishery, but possible causes can be inferred from the literature and known marine phenomena. The daily pattern in catch rates is probably similar to that observed for silver hake on Georges Bank (Edwards and Emery 1968; Bowman and Bowman 1980) in response to diel feeding migrations. The long period cycle is more difficult to explain. Long period cycles are suggestive of tidal influences on catchability, which have been claimed by skippers in the silver hake fleet (R. Haliday, Department of Fisheries and Oceans, Dartmouth, NS B2W 4W2, Canada, personal communication), but none of the principle lunar or solar tidal components are weekly in nature (Dietrich 1957). This could be the technological effect of gear efficiency that changes with the variation in tidal flow through the fortnightly tidal cycle (potentially minimal at 7-day intervals). Alternatively, an interaction between diel feeding migrations and fortnightly tidal cycles may be creating variation in catchability over a 7-day period, a potential relationship that can be seen by examining the product of a 14-day cycle and a 24-h cycle. Although further investigation is required to determine the source and true extent of these cycles, their potential presence remains a concern for the inference of local abundance from catch rates.

Mean catch rates varied significantly among vessels, but not to the extent that has been reported in some previous studies (e.g., Abrahams and Healey 1990). Hilborn (1985) suggested that selection for financial success would favor more uniformly skillful fishers in commercial fleets than in recreational fisheries. In the case of the silver hake fleet, for both Russians and Cubans, command of a vessel is assigned to a limited number of individuals based upon previous performance. Such stringent selection should minimize the variability in individual skills. Hilborn and Ledbetter (1985) estimated that vessel performance differences were approximately one third skill and two thirds chance among British Columbian purse seiners fishing for salmon; the significant role of chance events in fishing success for silver hake is illustrated by the distribution of catch rates among vessels observed in event A.

Ultimately, the proportion of variability in catch rates due to cycles seems small, and the variability in catch rates due to vessel interactions may be impossible to determine in the data provided by a typical fishery. However, the potential importance of these factors is underrepresented by catch and effort data alone. Long period cycles may not have a great impact on overall abundance estimates, but they may profoundly affect the accuracy of attempts to determine the spatial distributions and movements of fish from survey data. In contrast, the presence of interference in commercial fisheries is important because it will reduce the spatial variability of CPUE data rather than increase it. Fisheries models that incorporate fleet dynamics, such as those proposed by Hilborn and Walters (1987), are better suited for the evaluation of the potential impact of interference than an analysis of catch rates alone. However, it is imperative that analyses such as those presented here be developed to support the relevance of these models to commercial fisheries.

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Application of microsatellite DNA variation to estimation of stock composition and escapement of Nass River sockeye salmon (*Oncorhynchus nerka*)

Terry D. Beacham and Chris C. Wood

Abstract: Spawning escapements of individual Pacific salmon stocks returning to remote spawning locations throughout large river systems can in theory be estimated by mixed-stock analysis of appropriately weighted samples from test fisheries near the river mouth. However, the feasibility of this approach has usually been limited by practical difficulties in identifying closely related populations within the same watershed. Microsatellite DNA techniques offer new promise for overcoming these limitations as illustrated for sockeye salmon (*Oncorhynchus nerka*) in the Nass River of northern British Columbia. Variation at six microsatellite DNA loci (Omy77, Ots3, Ots100, Ots103, Ots107, and Ots108) was surveyed from about 1400 fish from nine stocks in the Nass River drainage as well as from 249 fish in a test fishery conducted in the lower river during 1996. Five stocks were surveyed in more than one year, and variation in allele frequencies among stocks was, on average, about 10 times greater than annual variation within stocks. Allele frequencies of stocks where the juveniles do not rear in lakes ("riverine" or "sea type") were more similar to each other compared with frequencies from lake-rearing stocks. Significant differences in allele frequencies were observed among most stocks at all loci. About 4.5% of observed variation over all loci was attributable to stock differentiation. Simulated mixed-stock samples suggested that the six microsatellite DNA loci surveyed should provide the ability to provide relatively accurate and precise estimates of stock composition when utilized for fishery management applications. The estimated proportion of Meziadin Lake sockeye salmon in the 1996 test fishery was about 73%, in close agreement with an estimate derived from direct tagging of fish in the test fishery.

Résumé : Il est possible en théorie d'estimer les échappées de géniteurs de chacun des stocks de saumon du Pacifique revenant vers des frayères éloignées dans les grands réseaux hydrographiques grâce à l'analyse de stocks mixtes à partir d'échantillons correctement pondérés obtenus par des pêches expérimentales menées près de l'embouchure des cours d'eau. Toutefois, la faisabilité de cette approche est généralement limitée par les problèmes pratiques que pose l'identification de populations étroitement apparentées dans un même bassin. Les techniques d'étude des microsatellites de l'ADN offrent de nouvelles possibilités de surmonter ces limitations, comme le montrent nos travaux sur le saumon rouge (*Oncorhynchus nerka*) de la Nass, dans le nord de la Colombie-Britannique. Nous avons examiné les variations à six locus de microsatellites (Omy77, Ots3, Ots100, Ots103, Ots107 et Ots108) chez environ 1 400 poissons appartenant à neuf stocks du bassin de la Nass, ainsi que chez 249 poissons capturés dans une pêche expérimentale menée en 1996 dans le cours inférieur de la rivière. Cinq stocks ont été étudiés sur plus d'un an, et la variation des fréquences des allèles entre les stocks était, en moyenne, environ 10 fois plus grande que la variation annuelle à l'intérieur des stocks. La comparaison des fréquences des allèles faisait ressortir plus de ressemblances entre les deux types de stocks dont les juvéniles ne grossissent pas dans les lacs (type lotique ou océanique) qu'entre ces deux groupes et les saumons de type lacustre. Des différences significatives dans les fréquences des allèles ont été observées chez la plupart des stocks à tous les locus. Environ 4,5% de la variation observée sur tous les locus était attribuable à la différenciation des stocks. La simulation d'échantillons de stocks mixtes semble montrer que les six locus de microsatellites examinés devraient permettre d'obtenir des estimations relativement exactes et précises de la composition des stocks quand on les utilise pour des applications à la gestion des pêches. La proportion estimée du saumon rouge du lac Meziadin dans la pêche expérimentale de 1996 était d'environ 73%, ce qui concorde avec une estimation tirée du marquage direct des poissons dans la pêche expérimentale.

[Traduit par la Rédaction]

Introduction

Estimation of the number of fish returning to spawn (escapement) is a key aspect of management and assessment of Pacific salmon. However, estimation of escapement in large remote watersheds containing riverine and glacial lake habitats can pose considerable practical difficulties. For example, the magnitude of sockeye salmon (*Oncorhynchus nerka*)

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production from some riverine and glacial lake habitats was not recognized until recently because of difficulties in directly enumerating spawning stocks that are sparsely distributed in turbid water throughout remote areas (Wood et al. 1987). In theory, it should be possible to estimate the relative escapements of individual spawning populations by mixed-stock analysis of appropriately weighted samples from test (assessment) fisheries near the river mouth. However, the feasibility of this approach has usually been limited by practical difficulties in identifying closely related populations within the same watershed. New techniques for screening genetic variation at highly polymorphic microsatellite DNA loci offer promise for overcoming these limitations because they frequently reveal differentiation among local populations not detected by analyses of mitochondrial DNA or allozymes.

Microsatellite DNA loci are abundant in all eukaryotic genomes and consist of tandem arrays of short (usually 2–4 base pairs (bp)) repeating sequences, flanked by regions of nonrepetitive DNA. Analysis of variation at microsatellite DNA loci requires only a small amount of tissue (nonlethal sampling), which can be conveniently stored in alcohol during field sampling. Provided suitable primers are available, specific loci are amplified with the polymerase chain reaction (PCR) and alleles routinely scored by gel electrophoresis on acrylamide gels. Microsatellite DNA loci have been reported to be highly variable among salmonid populations, with heterozygosity usually greater than that observed at protein electrophoretic and minisatellite DNA loci (Nelson et al. 1998). Microsatellite DNA variation in sockeye salmon has been previously demonstrated to be of potential value in stock identification of stocks in a local area (Beacham et al. 1998). This capability to detect differences among closely related populations can be of special value in mixed-stock analysis of test fisheries. One such application is in the Nass River in northern British Columbia.

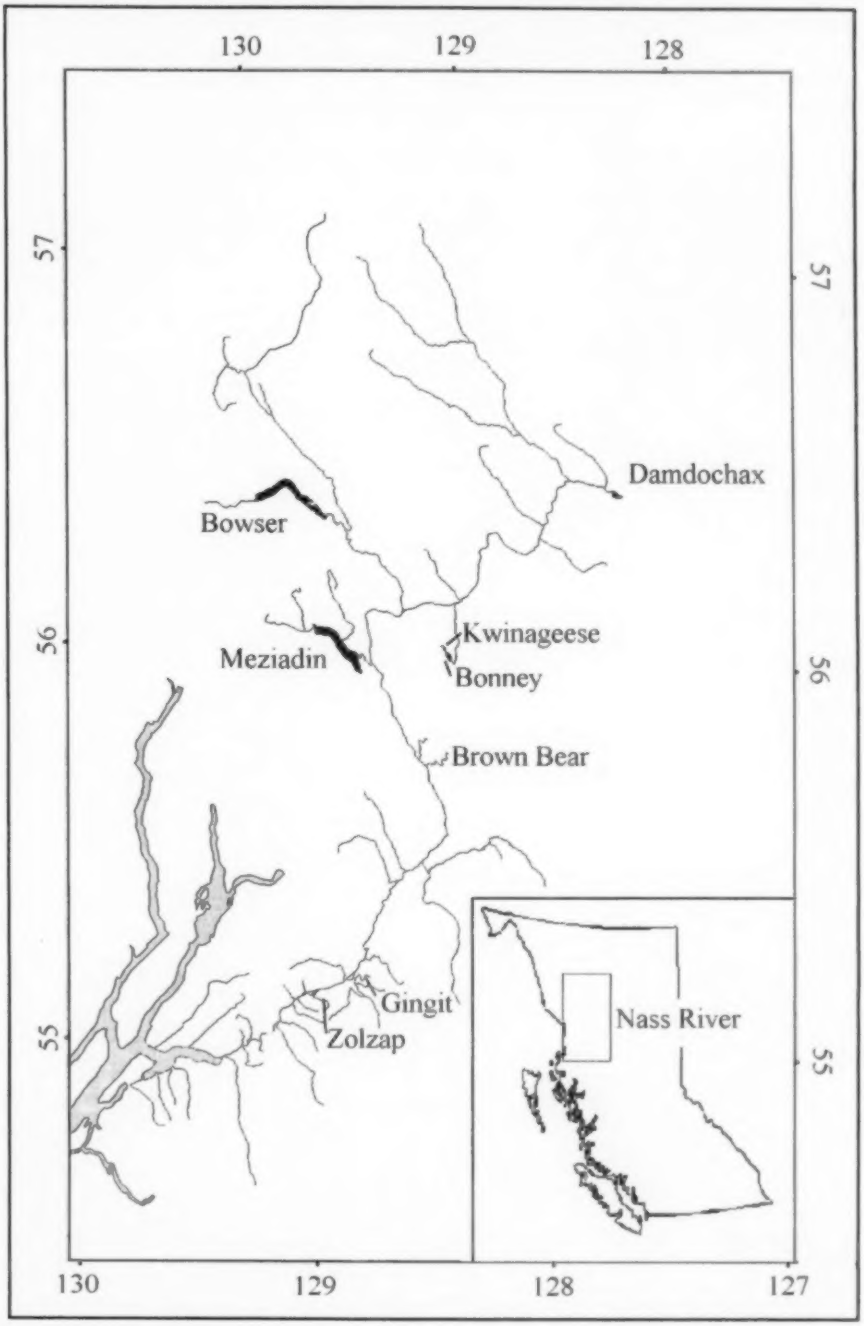
Sockeye salmon returning to the Nass River comprise an important group of stocks, with returns from 1991–1995 ranging from 300 000 to 1.4 million fish (Rutherford et al. 1994; Link and English 1996; Link and Gurak 1997). It is impossible to enumerate visually the spawning escapements in some of the stocks returning to glacially turbid lakes and rivers in the drainage. A test fishery has been conducted annually since 1963 in the lower Nass River (Todd and Dickson 1970), and the test fishery provides managers with an index of daily sockeye salmon escapement to the drainage (Southgate et al. 1990). As it is impossible to enumerate the escapements of all stocks visually, an alternative method of escapement estimation is desirable.

Less than 10 stocks in the drainage are thought to account for most of the returns (Fig. 1), with sockeye salmon returning to Meziadin Lake the dominant contributor to drainage escapement (Rutherford et al. 1994). The escapement of Meziadin Lake sockeye salmon is thought to be reliably determined at a fishway at Victoria Falls near the outlet of the lake. With the escapement of Meziadin Lake sockeye salmon known, it has been possible to estimate escapements to the other systems in the drainage based upon the known Meziadin Lake escapement and the relative proportions of the different stocks in the lower river test fishery, assuming representative sampling has occurred in the test fishery. Prior to 1992, the test fishery was conducted with gill nets (Todd and Dickson 1970; Southgate et al. 1990), but in 1992, fishwheels were introduced for salmon capture (Link et al. 1996), and by 1994 the gillnet test fishery was replaced with fishwheels and a mark–recapture program, which were thought to provide a more reliable estimate of sockeye salmon escapement (Link and English 1996).

Stock composition analysis in the test fishery using biological markers has centered upon using scale pattern analysis, freshwater age, a brain parasite, and protein electrophoretic variation (Rutherford et al. 1994). Freshwater age derived from scales differed among stocks, with age 2.* fish found largely in the two largest and glacially turbid lakes (Bowser and Meziadin lakes), but the proportion of age 2.* fish within these two stocks varied substantially among years. The presence of the myxosporean parasite *Myxobolus arcticus* was found to infect over 90% of Damdochax Lake fish and about 30% of Gingut Creek fish, with a low prevalence (<6%) observed in the other stocks (Rutherford et al. 1994). Protein electrophoretic analysis indicated that Meziadin Lake sockeye salmon were quite distinctive, but that there was little differentation among sockeye salmon from Bowser Lake and those from Bonney Creek and Kwinageese River in Fred Wright Lake (Rutherford et al. 1994).

The lower river test fishery is important in determining drainage escapement as well as escapement in Bowser Lake. The original procedure was to use scale pattern analysis to separate age 2.* fish in the test fishery to either Meziadin Lake or Bowser Lake origin (Todd and Dickson 1970). The escapement of Meziadin Lake sockeye salmon was determined from the fishway count and the proportion of age 2.* fish determined from sampling. With the number of age 2.* Meziadin fish thus determined, and the relative proportions of Bowser Lake and Meziadin Lake 2.* fish determined from the test fishery, it was possible to estimate the number of Bowser Lake 2.* fish. Escapement sampling for the relative proportions of age 1.* and 2.* fish allowed the number of 2.* fish to be expanded to an estimate of total escapement for Bowser Lake. Scale pattern analysis was reliable for separation of Bowser Lake and Meziadin Lake sockeye salmon (Todd and Dickson 1970; Rutherford et al. 1994), but greater differentitation among stocks was required to provide independent estimates of run timing and spawning escapement for each major sockeye salmon stock in the Nass River drainage. The use of data from five protein electrophoretic loci, the prevalence of *M. arcticus*, and freshwater age increased the ability to resolve stocks, with escapement estimated for five stocks from 1986 to 1992 (Rutherford et al. 1994). However, the inclusion of protein electrophoretic and parasite prevalence data required killing fish in the test fishery, which was not always considered practical. Use of scale pattern analysis continued for estimation of the Bowser Lake escapement, and in 1992, an annual mark–recovery program was initiated to provide a drainage-wide escapement estimate as well as an estimate of the proportion of Meziadin Lake sockeye salmon in the total escapement (Link and Gurak 1997). Especially in recent years, discrepancies in estimates of stock composition using scale pattern analysis and mark–recapture have occurred (Link and Gurak 1997), and thus,

Fig. 1. Locations in the Nass River drainage where sockeye salmon were sampled during 1987–1997.


130
129
128
57
56
55
Damdochax
Bowser
Meziadin
Kwinageese
Bonney
Brown Bear
Gingit
Zolzap
Nass River
130
129
128
127

we belived that it was opportune to investigate biological markers for stock identification that did not require lethal sampling for collection.

In the present paper, we evaluate variation at six microsatellite DNA loci and compare the level of variation among years within stocks with the level of differentiation among stocks. We then evaluate the utility of using microsatellite DNA variation for stock identification of Nass River sockeye salmon and then apply the technology to estimate stock composition in the 1996 test fishery and escapements and compare the results with those derived from scale pattern analysis and tagging.

Table 1. Number of fish sampled by year for baseline stocks, and number of fish included from the 1996 test fishery by week.

Stock	Years/dates	Number	Total
Bonney	1987, 1994, 1996	76, 81, 93	250
Gingit	1987, 1988, 1997	73, 93, 169	335
Kwinageese	1987	81	81
Meziadin	1987, 1996	100, 111	211
Damdochax	1987, 1994	100, 81	181
Bowser	1986, 1987, 1994	80, 72, 81	233
Zolzap	1996*, 1997	36, 24	60
Brown Bear	1997	40	40
Test fishery	June 12–18, 19–25	15, 14	
	June 26 – July 2	62	
	July 3–9, 10–16, 17–23, 24–30	18, 27, 28, 32	
	July 31 – August 6	36	
	August 7–13, 14–20, 21–27	10, 4, 2	
	August 28 – September 3	1	249

*Juveniles.

Table 2. Precision of estimation of allele size for a single fish analyzed repeatedly at each locus, with the fish run only once on each gel.

Locus	*N*	Allele size (bp)	Range
Ots3	51	74.20 (0.40)	74–75
	51	93.49 (0.51)	93–94
Omy77	70	104.47 (0.56)	104–106
	70	116.14 (0.52)	115–117
Ots107	70	109.89 (0.58)	109–111
	70	117.72 (0.59)	117–119
Ots108	48	111.85 (0.65)	111–114
	48	184.83 (0.83)	183–186
Ots100	67	158.67 (0.73)	157–160
	67	185.44 (0.91)	184–187
Ots103	68	174.96 (0.76)	173–176
	68	213.19 (1.37)	211–216

Note: *N* is the number of gels where allele sizes for the fish were estimated. SD given in parentheses.

Methods and materials

Collection of DNA samples and PCR

For the characterization of the baseline stocks prior to 1997, DNA was extracted from liver samples of adult sockeye salmon that had previously been collected for protein electrophoretic studies and frozen at –20°C. The only exception was the 1996 Zolzap Creek sample, which consisted of juveniles preserved in ethanol. The 1997 collections consisted of adult fin clips from the Zolzap Creek and Brown Bear Creek stocks preserved in ethanol. About 1300 fish were sampled from eight stocks, with six of the stocks sampled in two or more years (Table 1). About 0.3 g of tissue was placed in each well of a 96-well plate containing 0.2 mL of 5% chelex in TE buffer (10 mM Tris, pH 7.4, 1 mM EDTA, pH 8.0, 0.10 mg proteinase K/mL, and 0.1% SDS) and incubated for 15 min at 50°C and then incubated for an additional 15 min at 95°C. The supernatant from each well was collected and placed in a fresh 96-well plate and stored at –20°C. About 1 mL of this extract was required for each PCR.

Loci amplified via PCR were the dinucleotide repeats Omy77 (Morris et al. 1996) and Ots3 (Banks et al. 1999) and the tetranucleotide repeats Ots100 (Nelson et al. 1998), Ots103 (Beacham et al. 1998), Ots107 (Nelson and Beacham 1999), and Ots108 (Nelson and Beacham 1999). For all primer sets used in this study, PCR was conducted in 25-mL reactions containing 12 pmol (0.48 mM) each primer, 80 mM each nucleotide, 20 mM Tris, pH 8.8, 2 mM $MgSO_4$, 10 mM KCl, 0.1% Triton X-100, 10 mM $(NH_4)SO_4$, and 0.1 mg nuclease-free bovine serum albumin/mL. All PCR in this study was preceded by an initial denaturation step of 3 min at 94°C. All cycle extension (30 cycles for all loci except Ots108, which was 35 cycles) steps were for 60 s at 72°C and all cycle denaturation steps were for 20 s at 94°C. PCR of loci Omy77, Ots3, Ots100, Ots103, Ots107, and Ots108 was accomplished with an annealing temperature at 48, 50, 57, 55, 48, and 46°C, respectively. Annealing times were 30 s for Omy77 and Ots100 and 60 s for the other loci.

Gel electrophoresis and band analysis

PCR products were size fractionated on 16 × 17 cm nondenaturing polyacrylamide gels visualized by staining with 0.5 mg/mL ethidium bromide in water and illuminating with ultraviolet light. Nelson et al. (1998) provided a more complete description of gel electrophoretic conditions. All gels were run for 14–18 h at 65–70 V, with gels at 8% acrylamide for analysis of Ots100 and Ots103 and 10% acrylamide for analysis of Omy77, Ots3, Ots107, and Ots108. Twenty-nine lanes per gel were loaded, with one outside lane containing 1-kbp ladder DNA (GibcoBRL), three 20-bp marker lanes (Gensura Labs Inc., Del Mar, CA), one standard fish to determine precision of estimation of allele size, and 24 individual fish analyzed.

Gels were scanned at a 1024 × 1024 pixel density with a Kodak charge coupled device camera with low light capability and a yellow filter. Images were analyzed using BioImage Whole Band software (Millipore Corp. Imaging Systems, Ann Arbor, Mich.), with the size of the amplified microsatellite alleles reported to the nearest base pair based upon the molecular size grid created with the 20-bp markers.

As there was some uncertainity in estimation of allele size as determined from the 20-bp grid, we identified alleles on the basis of a binning procedure (Gill et al. 1990). Peaks in the allele frequencies were used to identify main alleles (Fig. 2), and bin widths generally corresponding to a repeat unit were set with the main allele occurring in the middle of the bin. Precision of estimation of allele size was evaluated with the standard fish analyzed for each locus.

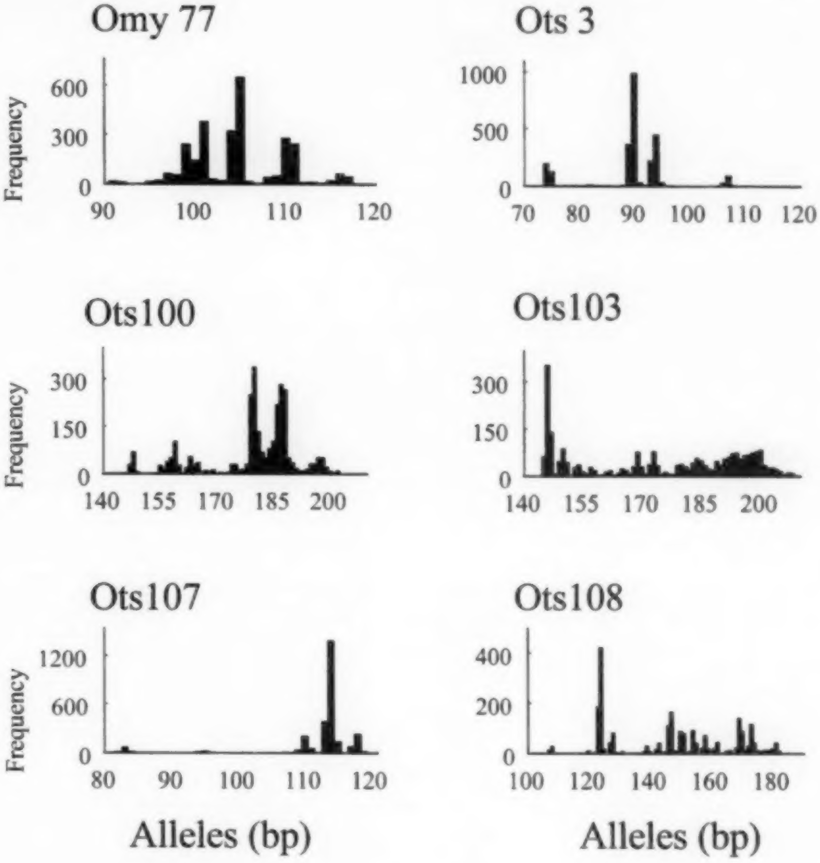
Precision of estimation of allele size

Standard deviations of the estimate of allele size for the heterozygous standard fish analyzed at each locus ranged from 0.40 to 1.37, with the larger alleles estimated with the least precision (Table 2). For Ots3, 100% of the estimated sizes for each allele spanned a 2-bp interval for both alleles. For the smaller allele at Omy77, 97% (68/70) of the estimated sizes of the allele were in a 2-bp interval, as were 93% of the estimated sizes of the larger allele. Estimated sizes of alleles of the standard fish that were analyzed at the other loci were all estimated within a 4-bp interval for alleles between 100 and 185 bp, with 85% of the estimated sizes of the larger allele at Ots103 (214 bp) within a 4-bp interval.

Data analysis

Each stock at each locus was tested for departure from Hardy–Weinberg equilibrium (HWE) using GENEPOP version 3.1 (Raymond and Rousset 1995) as was temporal stability of allele frequencies. For stocks sampled in more than one year, HWE was tested on samples pooled over years if no significant annual varia-

Fig. 2. Observed distribution of alleles (nearest bp) at the Omy77, Ots3, Ots100, Ots103, Ots107, and Ots108 loci for Nass River sockeye salmon.



tion in allele frequencies was observed. For those stocks in which significant annual variation in allele frequencies was detected at any locus, HWE was tested on an annual basis at all loci in the stock. In this case, the lowest probability of conformance to HWE in the annual tests was reported at the locus. Tests of genetic differentiation utilizing pairwise comparisions between all population pairs (36 pairs from nine populations) at each locus were also conducted using GENEPOP. The dememorization number was set at 1000, and 50 batches were run for each test with 1000 iterations per batch. Critical signficance levels for simultaneous tests were evaluated using sequential Bonferroni adjustment (Rice 1989). The Cavalli-Sforza and Edwards (1967) chord distance was used to estimate distance among populations. Estimation of components of allele frequencies among stocks and years (hierarchial gene diversity analysis) was determined with BIOSYS (Swofford and Selander 1981). Principal components were calculated with the PRINCOMP procedure in SAS (SAS Institute Inc. 1989). Identification of individuals to specific stocks was done with the DISCRIM procedure in SAS with a jackknife sampling procedure. Classification functions were developed using all fish sampled except the one to be classified, with each fish tested individually in turn. This procedure should reflect the accuracy expected when applied to new previously unsampled fish. Identification of individual fish was restricted to those fish where data were available at all six loci analyzed and the data for each fish summarized by 77 principal component scores from the original 108 possible alleles. The 77 principal components summarized all of the original observed variation.

Estimation of stock composition

The model of Fournier et al. (1984) was used to estimate stock composition, and genotypic frequencies were determined at each locus for each stock. Each locus was considered to be in HWE, and expected genotypic frequencies were determined from the observed allele frequencies and used as model inputs. Observed genotypic frequencies could be used, but expected genotypic frequencies are preferable, as there is some possibility of observing genotypes from a stock present in the mixture that has not been observed in the baseline samples. Each baseline stock was resampled with replacement in order to simulate random variation involved in the collection of the baseline samples before the estimation of stock composition of each mixture. If small stock samples are present in the baseline, estimated stock compositions for that stock will tend to have higher variances, and this was incorporated into the simulation analyses. Two mixture compositions were examined that would be typically expected in returning Nass River sockeye salmon in order to evaluate accuracy and precision of the estimated stock compositions. Hypothetical fishery samples of 200 fish were generated by randomly resampling with replacement the baseline stocks and adding the appropriate number of fish from each stock to the mixture. Estimated stock composition of the mixture was then de-

Table 3. Observed (H_o) and expected (H_e) heterozygosities, probability of conformance to Hardy–Weinberg equilibrium (HWE), and probability of homogeneity of allele frequencies among sampling years within stocks (years).

Stock	Trait	Omy77	Ots3	Ots100	Ots103	Ots107	Ots108
Bonney	H_o	0.699	0.325	0.790	0.811	0.452	0.749
	H_e	0.708	0.325	0.765	0.834	0.444	0.778
	HWE	0.055	0.161	0.190	0.050	0.090	0.043
	Years	0.026	0.314	0.000*	0.000*	0.129	0.561
Kwinageese	H_o	0.654	0.556	0.827	0.846	0.457	0.716
	H_e	0.635	0.570	0.739	0.885	0.414	0.798
	HWE	0.818	0.574	0.295	0.578	0.571	0.105
Meziadin	H_o	0.617	0.667	0.442	0.735	0.270	0.738
	H_e	0.679	0.683	0.436	0.775	0.291	0.780
	HWE	0.106	0.614	0.671	0.090	0.055	0.067
	Years	0.000*	0.004*	0.000*	0.000*	0.910	0.000*
Damodchax	H_o	0.793	0.587	0.634	0.871	0.508	0.804
	H_e	0.816	0.546	0.634	0.862	0.510	0.801
	HWE	0.388	0.893	0.323	0.044	0.045	0.323
	Years	0.051	0.051	0.103	0.218	0.072	0.730
Bowser	H_o	0.714	0.605	0.786	0.863	0.365	0.874
	H_e	0.743	0.591	0.792	0.859	0.352	0.859
	HWE	0.123	0.435	0.475	0.501	0.795	0.243
	Years	0.426	0.065	0.277	0.078	0.435	0.046
Gingit	H_o	0.760	0.439	0.707	0.878	0.422	0.882
	H_e	0.774	0.466	0.755	0.888	0.429	0.892
	HWE	0.207	0.050	0.049	0.048	0.019	0.000*
	Years	0.000*	0.500	0.001*	0.073	0.307	0.004*
Zolzap (j)	H_o	0.771	0.771	0.444	0.917	0.361	0.791
	H_e	0.753	0.725	0.520	0.818	0.401	0.783
	HWE	0.962	0.484	0.080	0.008	0.087	0.289
Zolzap (a)	H_o	0.771	0.783	0.542	0.875	0.292	0.682
	H_e	0.817	0.850	0.498	0.901	0.451	0.931
	HWE	0.199	0.793	0.541	0.906	0.038	0.014
Brown Bear	H_o	0.879	0.622	0.395	0.784	0.550	0.605
	H_e	0.850	0.767	0.439	0.891	0.496	0.877
	HWE	0.009	0.356	0.447	0.444	0.801	0.002*

Note: j, juveniles; a, adults.

termined, with the whole process repeated 100 times to estimate the mean and standard deviation of the individual stock composition estimates. Estimated stock composition of the test fishery was determined as a point estimate of the 249-fish sample, with standard deviations of individual stock estimates derived from bootstrap resampling of both the baseline stocks and the 249-fish mixture.

Test fishery

Fishwheels were used to conduct the 1996 test fishery, and numbered spaghetti tags (Floy model FT-4, Floy Tag and Manufacturing Co., Seattle, Wash.) were applied to sockeye salmon on a daily basis, with scales also collected daily (see details in Link and Gurak 1997). Daily tag releases at the fishwheels and recoveries at the Meziadin fishway were used to reconstruct the abundance of sockeye salmon in the lower river (Link and Gurak 1997). We then used the estimated total weekly sockeye salmon escapement past the test fishery to determine the appropriate number of fish to include in the test fishery sample that was analyzed. For example, if 10% of the total run was estimated to have passed by the test fishery in a particular week, then 10% of the 249 fish analyzed in our test fishery sample would have been picked randomly from fish sampled in that week. Numbers of fish included in the test fishery sample are outlined in Table 1. DNA was derived from a single previously collected scale for each fish included in the analysis, and samples within a week were randomly selected from the available pool of samples.

Results

Stock structure as indicated by variation in microsatellite DNA

Variation within stocks

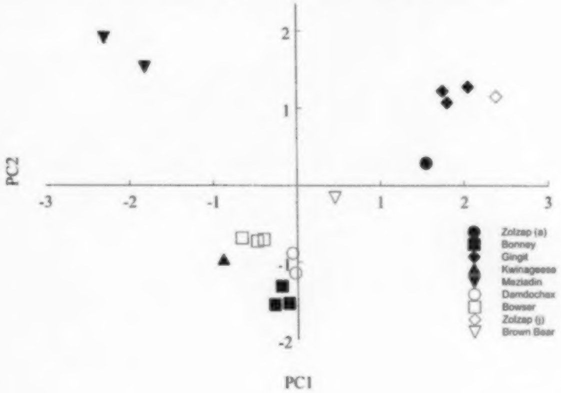
All six microsatellite loci examined were polymorphic in all stocks surveyed. Observed heterozygosity of the loci examined over all stocks was as follows: Omy77, 0.708 (stock range 0.62–0.88); Ots3, 0.52 (0.33–0.78); Ots100, 0.689 (0.40–0.83); Ots103, 0.846 (0.74–0.92); Ots107, 0.405 (0.27–0.55); Ots108, 0.783 (0.61–0.88). Significant departures from the expected Hardy–Weinberg distribution of genotypic frequencies were observed at the Ots108 locus in Gingit Creek and Brown Bear Creek sockeye salmon (Table 3). Significant annual variation in allele frequencies was observed at four loci in Meziadin Lake sockeye salmon, two loci in Bonney Creek fish, and two loci in Gingit Creek fish (Table 3).

Table 4. Pairwise Cavalli-Sforza and Edwards (1967) chord distance among nine stocks of Nass River sockeye salmon derived from six microsatellite DNA loci.

	Kwinageese	Meziadin	Damdochax	Bowser	Zolzap (j)	Zolzap (a)	Gingit	Brown Bear
Bonney	0.0081	0.0354	0.0127	0.0243	0.0525	0.0409	0.0358	0.0294
Kwinageese		0.0257	0.0136	0.0268	0.0555	0.0491	0.0432	0.0391
Meziadin			0.0299	0.0343	0.0570	0.0500	0.0408	0.0385
Damdochax				0.0169	0.0433	0.0396	0.0300	0.0356
Bowser					0.0524	0.0404	0.0386	0.0376
Zolzap (j)						0.0453	0.0249	0.0483
Zolzap (a)							0.0203	0.0209
Gingit								0.0191

Note: j, juveniles; a, adults.

Fig. 3. Plot of populations of Nass River sockeye salmon along the first two principal components. Annual samples within each population are indicated separately. a, adults; j, juveniles.



Variation among stocks

Genetic differentiation was observed among sockeye salmon stocks surveyed in the Nass River drainage at all six microsatellite loci examined. At the Omy77 locus, variation at Omy77^{98} ranged from 0.00 for Gingit Creek sockeye salmon to 0.37 for Bowser Lake fish, and the frequency of Omy77^{104} ranged from 0.14 for Bowser Lake fish to 0.53 for Kwinageese River fish (Appendix).

Substantial differentiation in allelic frequencies among stocks was observed at Ots3, with Meziadin Lake sockeye salmon most distinctive at this locus. For example, the frequency of Ots3^{74} for Meziadin Lake sockeye salmon was 0.31, with the next highest frequency of 0.17 observed in the Zolzap Creek adult sample (Appendix). The frequency of Ots3^{88} was 0.29 for Meziadin Lake fish whereas the frequency was 0.81 for Bonney Creek fish. At Ots100, Meziadin Lake sockeye salmon were again distinctive, with the frequency of Ots100^{179} 0.74 whereas the frequency of this allele in all other stocks was less than 0.21 (Appendix).

Genetic differentiation was observed among all stocks at the Ots103 locus. Meziadin Lake sockeye salmon were again relatively distinct, and the combined allele frequencies of Ots103^{178,182,186} for the non-lake-rearing Gingit Creek sockeye salmon (0.46), Zolzap Creek juveniles (0.38) and adults (0.33), and Brown Bear Creek fish (0.27) substantially greater than all other stocks surveyed (Bowser Lake fish next highest with a frequency of 0.15) (Appendix).

Four alleles accounted for in excess of 95% of the frequency of occurrence of all alleles at the Ots107 locus. Although there were a restricted number of alleles observed at this locus, frequency differences were still observed among stocks. The dominant Ots107^{113} allele ranged in frequency from 0.67 (Damdochax Lake) to 0.84 (Meziadin Lake) (Appendix). Variation in allelic frequencies at Ots108 was evident among stocks, with the frequency of Ots108^{126} for Gingit Creek sockeye salmon about 0.06 and that of the Zolzap Creek juveniles 0.03, substantially less than in the other Nass River stocks (Appendix). The frequency of Ots108^{177} was 0.25 in Meziadin Lake fish whereas the frequency in the other stocks was less than 0.10.

Most pairwise population comparisons for allele frequencies at each locus were significant ($P < 0.0001$), with some of the following exceptions. For Omy77, Bonney Creek versus Kwinageese River (both tributaries of Fred Wright Lake, $P = 0.054$), Gingit Creek versus Zolzap Creek juveniles ($P = 0.041$), and Kwinageese River versus Meziadin Lake ($P = 0.011$) were not significantly different. For Ots3, Kwinageese River versus Damdochax Lake ($P = 0.58$), Zolzap Creek adults versus Brown Bear Creek ($P = 0.57$), Gingit Creek versus Zolzap Creek juveniles ($P = 0.04$), and Kwinageese River versus Brown Bear Creek ($P = 0.04$) were not significant. For Ots103, Brown Bear Creek versus Zolzap Creek adults ($P = 0.15$) and the Bonney Creek and Kwinageese River comparison ($P = 0.015$) were not significant. For Ots107, Zolzap Creek adults versus Brown Bear Creek ($P = 0.92$) and Gingit Creek versus Zolzap juveniles ($P = 0.42$) were not significant. For Ots108, the only nonsignificant comparison was Brown Bear Creek versus Zolzap Creek adults ($P = 0.03$). Meziadin Lake was the most distinctive stock when adult allele frequencies were examined (Table 4).

Stock structure

Substantial genetic differentiation was observed among stocks in the Nass River drainage (Fig. 3), with 4.4% of total gene diversity averaged over all six loci attributed to variation among stocks (Table 5). At each locus, variation in allele frequencies among stocks was significantly greater than the annual variation within stocks and averaged over all loci was about 10 times greater. However, allele frequencies were similar among some of the population samples. For exam-

Table 5. Hierarchial gene diversity analysis of nine stocks of Nass River sockeye salmon for six microsatellite DNA loci evaluating the relative variation among sampling years within stocks and among stocks.

	Relative gene diversity		
Locus	Within stocks	Among years	Among stocks
Omy77	0.9363	0.0050	0.0587
Ots3	0.9145	0.0042	0.0813
Ots100	0.9074	0.0087	0.0838
Ots103	0.9537	0.0078	0.0385
Ots107	0.9757	0.0021	0.0222
Ots108	0.9549	0.0016	0.0435
All	0.9395	0.0052	0.0553

Fig. 4. Neighbor-joining dendrogram of relationships among sockeye salmon stocks from the Nass River. The distance measure used was the Cavalli-Sforza and Edwards (1967) chord distance. a, adults; j, juveniles.

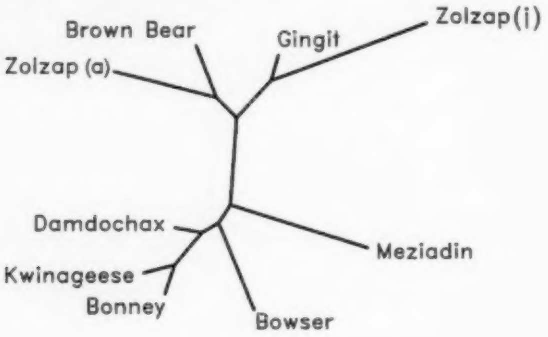


Table 6. Estimated percent composition of two mixtures of Nass River sockeye salmon in simulations using observed variation at six microsatellite DNA loci.

	Mixture 1		Mixture 2	
Stock	Correct	Estimated	Correct	Estimated
Zolzap (j)	0	0.0 (0.1)	0	0.0 (0.2)
Zolzap (a)	0	0.1 (0.3)	0	0.1 (0.2)
Brown Bear	5	3.6 (1.5)	0	0.2 (0.5)
Gingit	10	10.6 (1.1)	10	10.3 (0.9)
Σ riverine	15	14.4 (1.7)	10	10.7 (1.0)
Bonney	15	14.2 (2.4)	5	5.3 (1.7)
Kwinageese	0	1.7 (2.0)	5	5.1 (2.1)
Σ Fred Wright	15	16.0 (2.8)	10	10.4 (1.9)
Meziadin	50	48.4 (1.9)	70	68.0 (1.9)
Damdochax	5	6.7 (2.5)	5	5.8 (1.8)
Bowser	15	15.2 (1.9)	5	5.1 (1.1)

Note: Each mixture of 200 fish was generated 100 times with replacement and stock compositons of the mixtures estimated by resampling each baseline stock with replacement to obtain the original sample size and a new distribution of allele frequencies. The individual estimates for Zolzap, Brown Bear, and Gingit have been summed to provide an estimate for non-lake-rearing fish (Σ riverine). The individual estimates of Bonney and Kwinageese have been summed to provide an estimate for Fred Wright Lake (Σ Fred Wright). SD given in parentheses. j, juveniles; a, adults.

ple, the sample of juveniles from Zolzap Creek was similar to the Gingit Creek stock and it is possible, given that Zolzap Creek is downstream from Gingit Creek, that the juveniles may have moved downstream from Gingit Creek to seek other rearing areas. Sockeye salmon either spawning or rearing in rivers without access to lake habitat (Gingit, Zolzap, and Brown Bear) were more similar to each other than to other salmon in which the juveniles could rear in lakes (Fig. 4). Meziadin Lake sockeye salmon were distinct from other lake-rearing populations.

Estimation of stock composition

We evaluated whether the level of genetic differentiation observed among sockeye salmon stocks in the Nass River drainage can be applied to practical issues of stock identification. Two simulated test fishery mixtures were developed that could be representative of the relative abundance of spawning escapements. Meziadin Lake sockeye salmon currently comprise the most abundant stock, and the proportion of Meziadin Lake sockeye salmon was estimated with a high degree of accuracy and precision (Table 6), reflecting the relative genetic distinctiveness of this stock (Figs. 3 and 4). The proportion of Bowser Lake sockeye salmon in the mixtures also was well estimated when it comprised from 5 to 15% of the mixture. The two most genetically similar stocks, Bonney Creek and Kwinageese River, also could be distinguished reasonably well, but by summing the estimates, the precision of the estimate for the overall contribution of Fred Wright Lake was much improved. The proportion of non-lake-rearing stocks in the mixture could also be estimated reliably. The simulations indicated that the six microsatellite DNA loci surveyed could be used to provide relatively accurate and precise estimates of stock composition for fishery management applications in the Nass River drainage.

Identification of individuals

Some stock identification problems require the identification of individuals to specific stocks. In the Nass River drainage, the most distinctive stocks surveyed were Meziadin Lake and the non-lake-rearing stocks (Gingit, Zolzap, and Brown Bear), and accordingly, these stocks have among the highest correct classification rates, even though the individuals tested were not included in the development of the discriminant functions (Table 7). The greatest misidentifications occurred between Bonney Creek and Kwinageese River, both tributaries of Fred Wright Lake. However, nearly 75% of the fish from these two stocks were identified as originating from Fred Wright Lake. When a nine-stock classification was considered, 60% of all fish were correctly classified to stock. With this relatively high level of correct classification of individual fish to specific stocks, estimated stock compositions of mixtures of these fish should be accurate. About 85% of fish sampled from non-lake-rearing stocks were correctly identified as to origin. Although improvements can be made, such as adding additional loci to enhance discrimination, the microsatellite loci surveyed in our study provide the basis to identify fish to specific stocks.

Application to 1996 test fishery

Sockeye salmon returning to Meziadin Lake were estimated to account for about 73% of the fish sampled in the

Table 7. Percent correct classification of individual fish using jackknifed discriminant analysis for six stocks of Nass River sockeye salmon.

Stock	*N*	Bonney	Kwinageese	Σ FW	Meziadin	Damdochax	Bowser	Gingit	Zolzap (j)	Zolzap (a)	Brown Bear	Σ riverine
Bonney	236	**60.6**	15.3	**75.9**	1.3	9.3	5.5	0.9	0.9	0.9	5.5	8.2
Kwinageese	67	28.4	**43.3**	**71.7**	13.4	10.5	0.0	0.0	0.0	0.0	4.5	4.5
Meziadin	204	1.0	3.9	4.9	**87.3**	4.4	1.0	1.5	0.5	0.0	0.5	2.5
Damodchax	167	9.6	8.4	18.0	3.0	**56.3**	10.8	4.8	2.4	1.2	3.6	12.0
Bowser	187	2.7	2.1	4.8	4.3	11.2	**74.3**	1.1	0.0	0.0	4.3	5.4
Gingit	272	2.9	0.4	3.3	4.4	4.4	0.7	**69.9**	7.7	2.6	7.0	**87.2**
Zolzap (j)	34	0.0	0.0	0.0	0.0	0.0	0.0	17.7	**73.5**	0.0	8.8	**100.0**
Zolzap (a)	21	0.0	0.0	0.0	0.0	4.8	14.3	9.5	4.8	**38.1**	28.6	**81.0**
Brown Bear	31	9.7	3.2	12.9	16.1	9.7	6.5	22.6	3.2	3.2	**25.8**	**54.8**

Note: Σ FW refers to samples from Fred Wright Lake and is the sum of Bonney Creek and Kwinageese River percentages. The Σ riverine grouping is the sum of Gingit, Zolzap, and Brown Bear contributions. j, juveniles; a, adults. Values in bold would equal 100% if classification were perfect.

test fishery in 1996 (Table 8). With an observed count of 181 840 fish at the fishway, the total Nass River drainage escapement was thus estimated to be about 250 000 fish, with about 17 000 fish estimated for escapement to the glacially turbid Bowser Lake and with 13.5% of the returns or 34 000 fish estimated to spawn in areas without access to lake habitat for juvenile rearing. Escapement to Fred Wright Lake was estimated at 3400 fish, and that to Damodchax was estimated at 14 400 fish. However, both of these latter estimates are relatively uncertain, given the level of precision of the estimated stock compositions.

Discussion

Variation at microsatellite DNA loci has been very useful in determination of population structure in fish, particularly salmonids. Microsatellite loci are generally more variable than protein electrophoretic loci, with greater numbers of alleles and higher heterozygosities (Sánchez et al. 1996). Variation at microsatellite loci has been used to outline population differentiation on a broad continental scale (McConnell et al. 1995) or a more localized scale (García de León et al. 1997; Nielsen et al. 1997*b*). Our current survey was designed to investigate variation at microsatellite loci for sockeye salmon on a localized scale in the Nass River drainage and, if sufficient stock differentiation was available, to estimate stock composition in a test fishery. The high heterozygosities observed at the microsatellite loci in our study compared with protein electrophoretic loci (Wood et al. 1994) allowed substantial genetic differentiation to be detected and exploited for stock identification.

Significant annual variation in allele frequencies in Meziadin Lake sockeye salmon was observed at five of the six microsatellite loci surveyed. Sockeye salmon returning to Meziadin Lake to spawn pass though the fishway at Victoria Falls near the outlet of the lake and spawn in many tributaries to the lake and along the lakeshore itself. Previous surveys of variation of sockeye salmon spawning in the tributaries and along the lakeshore indicated that there was a signficant difference in allele frequencies at one protein electrophoretic locus between creek- and beach-spawning fish (Rutherford et al. 1994), indicative of some stock structure within the lake itself. Samples analyzed in our study were obtained from the fishway and would thus represent a mix of fish spawning in creek and lakeshore sites. Heterozygote deficiencies were observed at five of the six loci examined. These deficiencies suggest that the Meziadin Lake samples are in fact stock mixtures and that at least some of the annual variation observed at the microsatellites may reflect the year-to-year variation in the relative abundance of component stocks.

Some of the genetic differences observed among sockeye salmon stocks in the Nass River drainage may reflect differences in life history. Juveniles typically spend 1 year or more rearing in lakes before they smolt and migrate to the ocean, but some populations also utilize riverine and estuarine habitat for rearing when lake habitiat is unavailable (Wood et al. 1987). In the Nass River drainage, Gingit Creek, Zolzap Creek, and Brown Bear Creek sockeye salmon appeared as a distinctive lineage, and this stock complex is unique among the stocks surveyed in that the juveniles have

Table 8. Estimated percent stock composition of the 1996 test fishery sample and resulting escapement estimate (number of fish) for eight stocks of sockeye salmon in the Nass River drainage.

Stock	Composition	Escapement
Bonney	1.34 (1.62)	3 400
Kwinageese	0.00 (1.43)	0
Σ Fred Wright	1.34 (2.04)	3 400
Meziadin	72.63 (4.22)	181 840*
Damdochax	5.74 (4.59)	14 400
Bowser	6.75 (2.90)	16 900
Gingit	7.68 (2.82)	19 200
Zolzap (j)	0.87 (0.56)	2 200
Zolzap (a)	0.00 (0.61)	0
Brown Bear	4.99 (2.34)	12 500
Σ riverine	13.54 (3.05)	33 900
Drainage		250 440

Note: Summed allocations to Fred Wright composition and riverine stocks are as defined in Table 6. SD given in parentheses. j, juveniles; a, adults.

*Actual count from fishway.

no direct access to a lake for freshwater rearing. Over 99% of returning adults to Gingit Creek lack a freshwater annulus (Rutherford et al. 1994), which suggests that the juveniles migrate to the ocean during their first summer of rearing ("sea-type" sockeye salmon). The relative genetic distinctiveness of Gingit Creek sockeye salmon may reflect this juvenile rearing pattern (Wood 1995). In the Stikine River in northern British Columbia, non-lake-rearing sockeye salmon stocks were all genetically more similar to each other than to stocks where juveniles rear in lakes before smolting (Wood et al. 1987), reflecting the relationship between juvenile rearing strategy and genetic similarity. In the Nass River drainage, other sea-type stocks are known to spawn in the lower portion of the river, with those sockeye salmon in Gingit Creek having the largest spawning concentration (Rutherford et al. 1994). These other stocks (like Zolzap Creek) would likely be most similar genetically to Gingit Creek sockeye salmon, so the estimated contribution of Gingit, Zolzap, and Brown Bear creek stocks to the 1996 test fishery likely exceeded the actual contribution of these specific sites, but should be representative of the combined contribution of all sea-type and river-type sockeye salmon stocks in the drainage.

The significant genetic differentiation observed between fish from two tributaries of Fred Wright Lake (Bonney Creek and Kwinageese River) and the heterozygote deficiencies observed within samples from Meziadin Lake are consistent with the previous studies of genetic differentiation within these lakes (Rutherford et al. 1994). Although sockeye salmon have been reported to stray among tributaries within lakes (Hartman and Raleigh 1964; Varnavsky and Varnavskaya 1985), genetic differentiation among tributaries or spawning sites within lakes is not uncommon (Wilmot and Burger 1985; Varnavskaya et al. 1994; Wood et al. 1994; Burger et al. 1995).

The collection of appropriate and adequate samples of the stocks contributing to a mixed-stock fishery is critical to ensure reliable estimation of stock composition. Given the expense of sample collection from spawning stocks, particularly when a large number of stocks may contribute to a fishery, it is highly desirable that the characters used in stock identification be stable over time or that annual variation be much less than the differentiation among stocks. The choice of technique to use for estimation of stock composition in mixed-stock fisheries depends largely upon the relative differentiation among stocks of interest, the level of year-to-year variation within stocks, and the cost of analysis. Analysis of variation of genetic characters in salmonids has usually demonstrated that there is minor annual variation in protein electrophoretic (Grant et al. 1980; Beacham et al. 1987; Wood et al. 1994), minisatellite DNA (Taylor et al. 1994; Beacham et al. 1996), and microsatellite DNA loci (Nelson et al. 1998; Small et al. 1998). This is of interest because temporal changes in allele frequencies can affect mixed-stock fishery analysis (Waples 1990). In an analysis of microsatellite DNA variation in Atlantic salmon (*Salmo salar*) over some 60 years in one population, Nielsen et al. (1997*a*) reported that there was some change in allele frequencies over time, but individuals from samples 60 years apart clustered together when compared with the closest neighboring population and another reference population. Variation within a stock was less than the differentiation among stocks, and a similar pattern was observed among the Nass River sockeye salmon stocks surveyed in our study. In the case of Nass River sockeye salmon, the genetic variation attributable to stock differentiation was more than 10 times the variation attributable to annual variation within stocks, rendering annual variation in allele frequencies of little practical significance in estimation of stock composition in the drainage. Annual variation at six microsatellite loci accounted for 0.5% of total genetic variation, similar to the annual component of 0.3% of total variation observed at 32 allozyme loci (Rutherford et al. 1994). In particular, annual estimation of microsatellite allele frequencies in baseline populations would not be required for practical applications, although some level of monitering of allele frequencies over time would be prudent. For Nass River sockeye salmon, the observed level of differentiation among stocks, the relative stability of allele frequencies within stocks, and the reasonable cost of laboratory analysis suggest that microsatellite DNA variation is practical and effective in applying to problems of stock identification.

Simulated mixtures evaluated for Nass River sockeye salmon indicated that microsatellite DNA variation can be used to provide accurate and precise estimates of individual stocks in the mixtures. Precision of the estimated stock compositions of nine stocks of Nass River sockeye salmon was greater than that of three stocks of Barkley Sound sockeye salmon on the west coast of Vancouver Island analyzed at four microsatellite loci (Ots107 and Ots108 not surveyed) (Beacham et al. 1998). The level of precision of the estimated stock compositions depended upon the genetic distinctiveness of the stock, with the estimated compositions more precise for the more distinctive stocks. For example, when Gingit Creek and Fred Wright Lake fish each comprised 10% of a 200-fish mixture, the coefficient of variation (CV) was 10% for the estimated contribution of Gingit Creek, but 18% for Fred Wright Lake. The mean CV of stocks at 5% of the mixture was 34%, with a mean CV of 13% for stocks at 10%, a CV of 14% for stocks at 15%, a CV of 4%

for a stock at 50%, and a CV of 3% for a stock at 70% of the mixture.

Estimation of stock composition and classification of individuals to specific stocks are two distinct goals for fisheries management. In stock composition analysis, the characteristics of the whole sample are used to provide the most likely estimate. For classification of individuals, only the characteristics of the individual to be identified are used. As more information is available from a mixture than from a single individual, estimates of stock composition will always be more accurate than classifications of individual fish.

The most critical stock for providing a reliable estimate of stock composition in the test fishery is Meziadin Lake, as the fishway count is divided by the estimated proportion of Meziadin Lake sockeye salmon to provide an estimate of escapement to the Nass River drainage. Fortunately, Meziadin Lake sockeye salmon are quite distinctive when microsatellite allele frequencies are compared, particularly at Ots100. There have been differences in the estimated proportion of Meziadin Lake sockeye salmon between scale pattern analysis and direct tagging (Link and Gurak 1997). The estimated proportion of Meziadin Lake sockeye salmon in the 1996 test fishery derived from the microsatellite DNA was about 73%. The estimated proportion of Meziadin Lake sockeye salmon from scale pattern analysis was 61% and that from tagging was 73%, if one assumes a 15% differential removal of tagged fish due to net fisheries and handling-induced mortality (Link 1999). M.R. Link (LGL Ltd., 9768 Second Street, Sidney, B.C., personal communication) has suggested that a 15% differential removal of tagged fish is the most reasonable rate to apply to adjust the number of tagged fish at the fishwheels in order to determine the proportion of Meziadin Lake fish. There was clearly a close agreement between the estimate derived from microsatellite DNA and that from tagging. Further comparisons should be conducted to evaluate whether there is continued close agreement between the proportion of Meziadin fish derived from microsatellite DNA and that from tagging analyses.

Microsatellite DNA variation provided a marked increase in precision of estimated stock compositions over those derived from applying a combination of variation at four allozyme loci, the frequency of occurrence of the brain parasite *M. arcticus*, and freshwater age. For example, in a comparison of precision of estimated stock compositions from samples of about 250 fish, and with Bowser Lake sockeye salmon estimated to have comprised less than 10% of the sample, the standard deviation (SD) of the estimated Bowser Lake contribution was 2.9 from six microsatellite loci (current study) and 7.4 from the combination of four allozyme loci, the brain parasite, and freshwater age (Rutherford et al. 1994). Similarly, the standard deviation of the Bonney Creek component (both <5%) was 1.6 from microsatellites and 6.2 from the combination. In the specific case of Nass River sockeye salmon, microsatellite DNA loci provided estimates of stock composition of higher precision at the same mixture sample size than those derived from the combination of allozyme loci, the brain parasite, and freshwater age and presumably higher accuracy than those derived from scale pattern analysis. Although we have reported only our study of microsatellite DNA variation in Nass River sockeye salmon in the current paper, it is clear from other analyses in our laboratory that variation at microsatellite DNA loci and major histocompatibility complex loci provides an effective and practical means for discriminating among salmonid stocks.

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Appendix. Observed allele frequencies at six microsatellite loci for eight stocks and one test fishery of Nass River sockeye salmon.

Allele	Bonney	Gingit	Kwinageese	Meziadin	Damdochax	Bowser	Zolzap (j)	Zolzap (a)	Brown Bear	Test fishery
Omy77										
N	247	323	81	209	179	224	35	23	37	249
90	0.000	0.000	0.000	0.000	0.034	0.002	0.000	0.000	0.000	0.004
92	0.000	0.000	0.000	0.002	0.017	0.000	0.000	0.000	0.000	0.000
94	0.010	0.028	0.000	0.000	0.000	0.000	0.000	0.152	0.000	0.010
96	0.040	0.025	0.006	0.019	0.036	0.040	0.000	0.130	0.135	0.038
98	0.093	0.000	0.049	0.022	0.098	0.371	0.000	0.109	0.054	0.064

Appendix (*continued*).

Allele	Bonney	Gingit	Kwinageese	Meziadin	Damdochax	Bowser	Zolzap (j)	Zolzap (a)	Brown Bear	Test fishery
100	0.126	0.215	0.093	0.175	0.209	0.297	0.329	0.152	0.095	0.215
102	0.004	0.012	0.006	0.022	0.034	0.000	0.000	0.000	0.000	0.032
104	0.455	0.308	0.525	0.490	0.302	0.141	0.171	0.109	0.324	0.386
106	0.008	0.008	0.000	0.007	0.003	0.002	0.000	0.000	0.000	0.002
108	0.000	0.142	0.000	0.000	0.006	0.000	0.143	0.022	0.027	0.028
110	0.237	0.232	0.278	0.218	0.168	0.078	0.357	0.283	0.324	0.187
112	0.002	0.009	0.006	0.024	0.000	0.000	0.000	0.000	0.000	0.008
114	0.000	0.009	0.000	0.010	0.008	0.002	0.000	0.022	0.000	0.018
116	0.025	0.011	0.037	0.012	0.087	0.067	0.000	0.022	0.041	0.018
Ots3										
N	249	313	81	210	179	205	36	24	38	249
74	0.014	0.027	0.074	0.310	0.053	0.098	0.014	0.167	0.092	0.281
82	0.010	0.000	0.006	0.005	0.000	0.005	0.000	0.000	0.026	0.004
86	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
88	0.813	0.674	0.611	0.288	0.628	0.510	0.569	0.688	0.737	0.351
93	0.070	0.290	0.198	0.371	0.215	0.373	0.403	0.146	0.118	0.345
96	0.000	0.009	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000
99	0.000	0.000	0.000	0.000	0.000	0.000	0.014	0.000	0.000	0.000
103	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.021	0.000	0.000
105	0.092	0.000	0.111	0.026	0.101	0.015	0.000	0.021	0.026	0.020
Ots100										
N	248	323	81	208	175	224	35	23	33	249
142	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002
146	0.111	0.000	0.160	0.012	0.011	0.007	0.000	0.000	0.030	0.018
154	0.060	0.065	0.056	0.000	0.003	0.000	0.186	0.348	0.061	0.008
158	0.056	0.105	0.031	0.010	0.034	0.076	0.000	0.109	0.136	0.024
162	0.030	0.056	0.031	0.046	0.043	0.067	0.214	0.087	0.121	0.041
166	0.002	0.023	0.025	0.022	0.011	0.011	0.000	0.022	0.030	0.012
170	0.002	0.014	0.006	0.000	0.003	0.002	0.000	0.000	0.000	0.002
174	0.042	0.102	0.000	0.038	0.023	0.069	0.086	0.022	0.030	0.041
179	0.129	0.198	0.185	0.740	0.189	0.158	0.100	0.174	0.212	0.620
184	0.433	0.381	0.438	0.108	0.566	0.346	0.400	0.130	0.258	0.195
190	0.046	0.051	0.012	0.010	0.020	0.020	0.014	0.109	0.015	0.028
195	0.083	0.002	0.056	0.014	0.080	0.217	0.000	0.000	0.106	0.010
200	0.004	0.000	0.000	0.000	0.017	0.027	0.000	0.000	0.000	0.000
Ots103										
N	249	333	81	211	178	226	36	24	37	249
144	0.307	0.068	0.296	0.377	0.132	0.117	0.111	0.000	0.122	0.307
148	0.078	0.012	0.111	0.055	0.076	0.115	0.000	0.021	0.027	0.077
152	0.070	0.002	0.049	0.005	0.006	0.049	0.000	0.021	0.014	0.010
156	0.000	0.053	0.000	0.005	0.028	0.000	0.333	0.000	0.000	0.016
160	0.020	0.036	0.025	0.005	0.006	0.004	0.000	0.021	0.068	0.008
164	0.006	0.051	0.019	0.009	0.028	0.038	0.014	0.021	0.000	0.012
168	0.070	0.068	0.037	0.033	0.079	0.042	0.014	0.146	0.162	0.041
172	0.034	0.078	0.111	0.118	0.084	0.009	0.083	0.063	0.041	0.096
178	0.024	0.149	0.025	0.009	0.020	0.004	0.208	0.104	0.095	0.020
182	0.028	0.192	0.012	0.059	0.039	0.082	0.042	0.063	0.108	0.051
186	0.006	0.117	0.012	0.036	0.011	0.064	0.125	0.167	0.068	0.083
190	0.084	0.101	0.093	0.242	0.118	0.184	0.056	0.188	0.203	0.185
196	0.203	0.063	0.148	0.036	0.275	0.250	0.014	0.104	0.081	0.085
202	0.062	0.012	0.062	0.012	0.090	0.042	0.000	0.042	0.014	0.008
208	0.006	0.000	0.019	0.000	0.008	0.000	0.000	0.021	0.000	0.002
214	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.021	0.000	0.000

Appendix (*concluded*).

Allele	Bonney	Gingit	Kwinageese	Meziadin	Damdochax	Bowser	Zolzap (j)	Zolzap (a)	Brown Bear	Test fishery
Ots107										
N	250	335	81	211	181	230	36	24	40	249
81	0.044	0.064	0.037	0.009	0.036	0.009	0.083	0.083	0.050	0.018
93	0.004	0.000	0.031	0.040	0.000	0.000	0.000	0.000	0.000	0.033
101	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.002
105	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.002
109	0.068	0.036	0.080	0.033	0.169	0.196	0.042	0.063	0.063	0.043
113	0.724	0.690	0.753	0.836	0.666	0.780	0.764	0.729	0.688	0.799
117	0.158	0.199	0.099	0.078	0.130	0.013	0.111	0.125	0.175	0.102
121	0.000	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000
125	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
129	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.000
133	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Ots108										
N	241	311	67	210	179	231	34	22	38	195
122	0.002	0.013	0.000	0.000	0.003	0.000	0.000	0.068	0.013	0.005
126	0.400	0.063	0.313	0.326	0.330	0.240	0.029	0.091	0.211	0.385
130	0.058	0.027	0.030	0.102	0.014	0.076	0.000	0.023	0.000	0.062
133	0.008	0.000	0.007	0.000	0.000	0.000	0.000	0.000	0.013	0.000
137	0.000	0.000	0.000	0.000	0.000	0.002	0.000	0.023	0.000	0.000
141	0.002	0.006	0.045	0.002	0.003	0.058	0.015	0.091	0.013	0.013
145	0.015	0.096	0.015	0.007	0.053	0.006	0.353	0.091	0.000	0.026
149	0.118	0.080	0.254	0.136	0.142	0.147	0.059	0.091	0.158	0.090
153	0.178	0.029	0.119	0.038	0.073	0.048	0.015	0.091	0.132	0.036
156	0.066	0.113	0.022	0.002	0.011	0.134	0.221	0.136	0.132	0.008
160	0.041	0.140	0.022	0.021	0.053	0.017	0.015	0.114	0.066	0.031
164	0.002	0.159	0.000	0.007	0.025	0.000	0.088	0.045	0.026	0.005
168	0.006	0.029	0.000	0.012	0.014	0.002	0.000	0.000	0.000	0.008
172	0.100	0.061	0.142	0.048	0.237	0.162	0.206	0.068	0.145	0.095
177	0.004	0.100	0.030	0.300	0.036	0.056	0.000	0.068	0.053	0.223
182	0.000	0.080	0.000	0.002	0.003	0.050	0.000	0.000	0.026	0.008
187	0.000	0.006	0.000	0.000	0.003	0.000	0.000	0.000	0.013	0.008
192	0.000	0.007	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Note: Alleles have been designated by the lower limit of the bin. *N* is the number of fish scored at each locus in each stock. j, juveniles; a, adults.

Light and diel vertical migration: spectral sensitivity and light avoidance by *Mysis relicta*

Gideon Gal, Ellis R. Loew, Lars G. Rudstam, and Ali M. Mohammadian

Abstract: Ambient light levels determine the extent of diel vertical migration of many species including mysid shrimps. Light levels perceived by an organism depend on the intensity of light at the surface, the extinction of light through the water, and the sensitivity of the organism's light receptors. Each of these processes has spectral characteristics that should be taken into account when measuring perceived light levels. We used microspectrophotometry to determine that *Mysis relicta* has a single pigment with the characteristics of rhodopsin based on vitamin A_1 and a peak sensitivity of 520 nm. Similar to the use of the lux (scaled to human vision), we give ambient light levels scaled to the mysid's visual spectrum in mylux units. Mysid distributions were observed with acoustics around two artificial light sources in Cayuga Lake, New York. Mysids avoided light levels of 3.4×10^{-7} to 2.1×10^{-6} mylux. Similar light levels limited their vertical distributions during the night in Lake Ontario and during the day in Cayuga Lake. Of standard light sensors available, lux meters are more appropriate than photosynthetically active radiation meters for determining light levels perceived by mysids.

Résumé : L'éclairement détermine l'amplitude des migrations verticales nycthémérales chez de nombreuses espèces, notamment les mysis. Le niveau d'éclairement perçu par un organisme dépend de l'intensité de la lumière à la surface, de l'atténuation de la lumière dans l'eau et de la sensibilité de ses photorécepteurs. Chacun de ces processus possède des caractéristiques spectrales qui doivent être prises en compte quand on mesure les niveaux de lumière perçus. À l'aide de la microspectrophotométrie, nous avons déterminé que *Mysis relicta* possède un pigment unique présentant les caractéristiques de la rhodopsine, d'après le profil de la vitamine A_1 et la présence d'un pic de sensibilité de 520 nm. Au lieu du lux (unité à l'échelle de la vision humaine), nous avons établi l'unité mylux, à l'échelle du spectre visuel de la mysis, pour faire état des niveaux ambiants d'éclairement. Nous avons observé la distribution des mysis par une technique acoustique autour de deux sources de lumière artificielle, dans le lac Cayuga (New York). Les mysis évitaient les éclairement de plus de $3{,}4 \times 10^{-7}$ à $2{,}1 \times 10^{-6}$ mylux. Des niveaux semblables d'éclairement limitaient leur distribution verticale pendant la nuit dans le lac Ontario et pendant le jour dans le lac Cayuga. Parmi les capteurs de lumière standard, le luxmètre convient mieux que le capteur de rayonnement photosynthétiquement actif pour mesurer le niveau d'éclairement perçu par les mysis.

[Traduit par la Rédaction]

Introduction

The opossum shrimp, *Mysis relicta*, is a freshwater member of a mainly marine group of small crustaceans in the family Mysidaceae. It is a benthic–pelagic invertebrate that inhabits offshore, hypolimnetic waters. Mysids avoid light, remaining in deep water during the day and moving up to the epilimnion at night (Beeton and Bowers 1982). The vertical extent of migration is believed to be primarily limited by light levels and temperature (Teraguchi et al. 1975; Moen and Langeland 1989). Such diel vertical migration is a common behavior in marine and freshwater zooplankton and is generally explainable as a trade-off between the need to minimize predation risk and the need to maximize food intake.

The light level an organism can perceive (and is therefore able to avoid) depends both on the light intensity at different wavelengths and the spectral sensitivity of the eye. The wavelength-specific light intensity at depth, in turn, depends on both the incident light level at the surface and the spectral properties of the water. None of the normally used light sensors (radiometer measuring light in watts per square metre per second, photosynthetically active radiation (PAR) measuring light in micromoles of photons per square metre per second, or photometer measuring light in lux) may give light levels related to what an animal perceives because the spectral sensitivity of the organism likely differs from the spectral response of the sensor. For example, Teraguchi et al. (1975) and Rudstam et al. (1989) studied the vertical distribution of mysids in the water column and estimated the light levels (in lux) at the leading edge of the mysid layer. Teraguchi et al. (1975) found the leading edge to be associated with a narrow range of about 0.02–0.05 lx, while *Mysis mixta* was found to avoid light levels of 10^{-4} lx (Rudstam et al. 1989). However, lux may not be the appropriate measure

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of light levels perceived by mysids. When using any standard sensor, care must be taken in applying conclusions to other water bodies and organisms with different spectral sensitivity.

To correctly measure light levels perceived by an organism, we need (*i*) spectroradiometric information of the available light, (*ii*) wavelength-specific extinction coefficient values for the water column, and (*iii*) the spectral sensitivity of the organism. Studies on mysid spectral sensitivity have been conducted on animals from Lakes Huron and Michigan by measuring reaction time to light of varying wavelengths (Beeton 1959) and on animals from several localities in Finland and the Baltic Sea by using recordings of electroretinograms (Lindström and Meyer-Rochow 1987; Lindström et al. 1988). Lindström and co-workers reported peak sensitivities at 505, 550, and 570 nm for different populations and suggested that variation in peak spectral sensitivity can be attributed to differences in optical properties of the different water bodies. Here, we use microspectrophotometry to measure the spectral sensitivity of *M. relicta* from Cayuga Lake, New York.

The underlying hypothesis for this study was that the extent of *M. relicta* diel vertical migration is restricted by light levels. We argue that the limiting light levels should be determined by measuring light perceived by the organism. We therefore combine laboratory and field approaches to test our hypothesis. These included (*i*). measuring the spectral sensitivity of visual pigments in the mysid eye, (*ii*) developing a photometric unit for mysids (the "mylux") using the spectral sensitivity of the mysid eye (analogous to lux), (*iii*) determining the light levels, in mylux units, avoided by mysids in in situ experiments, and (*iv*) comparing these values with light levels at the upper levels of the mysid layer observed during diel vertical migration (night and day) in Cayuga Lake and Lake Ontario, New York.

Methods

Visual pigment analysis

Mysids used for microspectrophotometry were collected from Cayuga Lake. Animals were collected at night on several occasions from a depth of 35 m with the use of a 1-m-diameter conical net with a 0.5-mm mesh. A plastic bag was placed in the cod-end prior to collection in order to minimize water flow and potential damage to mysid specimens. Upon retrieval, mysids were transferred to an aerated cooler under the illumination of a flashlight. They were maintained in the laboratory at 4–6°C under dark conditions for several weeks before being used. On the day of examination, the mysids were exposed for a very short period of time (<5 min) to light when they were moved to a darkened cooler, where they were kept for a minium of 4 h before use. Preliminary measurements showed that this treatment did not cause appreciable bleaching of the visual pigment. The mysids used for analysis were 8–13 mm in length.

Microspectrophotometric measurements

Eyes from the dark-adapted mysids were isolated in a standard buffer (0.1 M phosphate at pH 7.4 with 6% sucrose) and macerated with tungsten needles to isolate the rhabdoms. A total of 11 rhabdoms were analyzed. All handling was conducted under a 15-W tungsten lamp with either a Kodak No. 2 darkroom filter (dim red light) or a Kodak No. 11 darkroom filter (infrared light) using image converters (FJW, Inc.).

The microspectrophotometer is a computer-controlled, single-beam instrument described in Loew and Wahl (1991). All procedures for visualization, lining up, and recording were done in the infrared. Data were collected at 1-nm intervals scanning from 750 to 350 nm and back to 750 nm. Comparisons of these scans give a good indication of overfiltering and bleaching, if any. All spectra ultimately saved and analyzed consisted of the even-nanometre data from the down-scan combined with the odd-nanometre data from the return scan. A digital Fourier filtering subroutine ("smooft", Press et al. 1989) was applied to the data to remove high-frequency noise. The filtering coefficient used (15) produced no appreciable broadening or phase shift as seen by overlaying the filtered and unfiltered data.

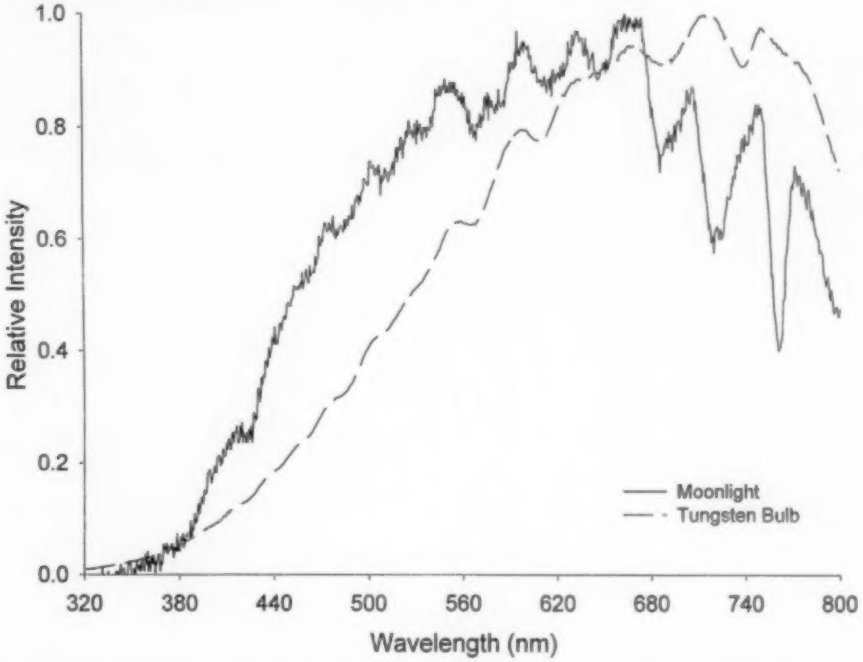
The template fitting procedure used a variation of the normalized optical density versus normalized frequency method (MacNichol 1986). The maximum absorbance in the raw data was determined by differentiation of the best fit Gaussian to the top 40 data points. This maximum value was used to normalize the data, which were then transformed from wavelength to normalized frequency. Best fit, first-order linear equations were obtained for the data. These equations were solved to allow template fitting with equations derived from the doubly normalized rhodopsin data of Partridge and DeGrip (1991) and Bridges' (1967) porphyropsin data using a commercial program (Tablecurve, Jandel Scientific). These equations were solved for 70 values previously calculated from the raw data. After transformation back to wavelength, estimates of λ_{max} for both vitamin A_1 and A_2 chromophores were obtained using each calculated value. Acceptance of calculated λ_{max} and selection of curves for further consideration were made by visual inspection of calculated template ("nomogram") curves overlaid on the normalized raw or filtered data. Since the machine wavelength error was ±1.0 nm, all visual pigment values are reported to the nearest integer.

Light avoidance experiments

On July 25, 1995, field experiments were conducted in Cayuga Lake. The objective of these experiments was to determine the degree of avoidance that mysids display in the presence of an artificial light source. A light source (bulbs of different power) was lowered during evening hours to the expected depth of the mysid scattering layer (about 15 m). The light was connected by cable to a standard marine battery on a small boat directly above it. Two different standard automotive brake bulbs were used: a T-57 bulb (3.36 W) and a T-89 bulb (7.54 W). The light apparatus consisted of a 0.5-in. (12.7-mm) PVC pipe and an automotive brake light socket and was suspended vertically.

Data on the mysid distribution were collected using a digital 420- kHz acoustic system (Biosonics, Inc., half-power beam angle 6°, ping rate 2 pings·s^{-1}). The acoustic sampling procedure was identical for each of the bulbs tested; once the light source was suspended (with the light off), we waited for a period of 30–60 min and then conducted several series of asterisk-shaped surveys over the region of the light source. Each asterisk (series) consisted of three transects, each originating at different positions and traversing as closely as possible to the light source. In order to determine if there was any effect of the apparatus on the mysid distribution, two series of acoustic transects were carried out while the light was off. These series were followed by two additional series with the light on (a total of four series). Between the light-off and light-on transects, we collected stationary acoustic data (both before and after turning the light on) immediately above the light source. Net samples were also taken at this time. After completing the sampling with the T-57 bulb, it was replaced with the T-89 bulb. The sampling procedure was then repeated.

Fig. 1. Normalized spectral output for two light sources: a tungsten bulb identical to those used in the light experiments and a normalized spectrum of the moonlight and starlight spectral output for a full moon and clear night in Ithaca, N.Y. (September 17, 1997). The tungsten bulb normalized spectral output was indistinguishable between bulbs examined (3.36 and 7.54 W). A value of 1 corresponds to the maximum absolute intensity.



Emission spectra for the tungsten brake bulbs were measured using a Princeton Applied Research OMA III scanning spectroradiometer. Calibration for both energy and quanta was done using a calibrated irradiance source (Gamma Scientific) traceable to the National Bureau of Standards (NBS) (now known as the National Institute of Science and Technology (NIST)). Measurements were conducted for voltages ranging between 10 and 12 V. The normalized spectral output can be seen in Fig. 1.

Two sources of spectroradiometric data were available to us. (*i*) On a single occasion (June 20, 1996), a Licor spectroradiometer (LI-1800 UW) was used to collect wavelength-specific light attenuation in Cayuga Lake. Data were collected for the PAR range (400–700 nm) down to the limit of detection by the instrument (23 m). Irradiance between 400–410 and 650–700 nm was too low to calculate extinction coefficient values (k_{PAR}) for these wavelengths, and mysids are not sensitive to the latter wavelengths (see below). (*ii*) Jerome et al. (1983) presented spectral irradiation extinction coefficients for six values of k_{PAR} for Lake Ontario (their fig. 4*b*). Peak spectral irradiances in Cayuga Lake (Fig. 2) were found between 560 and 564 nm for measurements between 14 and 23 m. Comparison of the Cayuga Lake spectroradiometer data and the Lake Ontario data for the same k_{PAR} showed no significant differences (K-S and sign test, $p > 0.5$) between the Cayuga Lake data and the appropriate curve ($k_{\mathrm{PAR}} = 0.4$) from Lake Ontario. Since the data reported by Jerome et al. (1983) included a wide range of k_{PAR} values, we used their data for the remainder of the study.

We estimated the light intensity perceived by the mysids at different distances from the light source by combining the available information on the light source, the surrounding medium, and the mysid visual spectrum. Light intensities of all wavelengths (400–650 nm) were calculated for 10.5 V (the expected voltage at the bulb) and extended to various ranges from the light source (described below, eq. 2) accounting for the spherical spreading of the light (by applying a multiplier of $(4\pi r^2)^{-1}$). Extinction coefficient values were wavelength specific and based on the spectral irradiance extinction coefficients. The available light at each wavelength was then scaled to the normalized mysid visual spectrum to give light levels in mylux (see below). Finally, we compared the available light with the acoustically observed mysid distribution and estimated the light levels at the distance from the light where mysids were first observed.

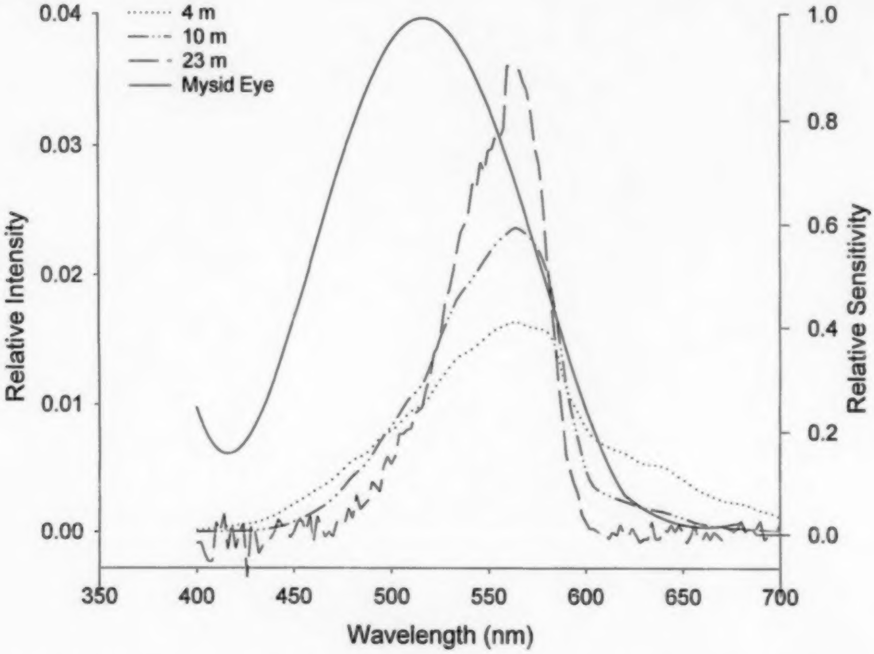
Acoustic analysis

Acoustic backscattering data were processed by using echo-integration (Visual Analyzer, V. 2.0, Biosonics, Inc.). Results of the echo-integration are given as s_{v} (volume backscattering coefficient, square metres per cubic metre), which is the ratio of the intensity backscattered by a unit volume to the intensity of the incident acoustic wave. The s_{v} values were averaged and converted to the logarithmic form S_{v} as defined below:

$$(1) \qquad \bar{S}_{\mathrm{v}} = 10\log_{10}(\bar{s}_{\mathrm{v}}) = 10\log_{10}\left(\frac{\sum s_{\mathrm{v}}}{n}\right)$$

We analyzed about 100–150 pings before and 100–150 pings after passing the position of the light source for each transect. In cases in which the light source was not visible on the echogram, its position was determined from the time noted when we passed the light source boat. The data were then segmented into 100 horizontal bins ("reports") of equal length and 10 depth strata between 12 and 22 m (the light was at 15 m). The reports were on average 1.3 s (SD = 0.13 s) long, the average boat speed was 0.8 m·s^{-1}, and the distance traveled per report was 1.05 ± 0.11 m. The acoustic beam

Fig. 2. Spectroradiometer data collected in Cayuga Lake, June 1996. Relative irradiance is the proportion of available light for each wavelength at three depths (4, 10, and 23 m). The total relative irradiance for a given depth is equal to 1. The mysid spectral sensitivity curve is plotted for comparison.



is conical, with a half-power beam width of about 1.6 m at a range of 15 m from the transducer. Therefore, there was always some spatial overlap between reports. Vertical resolution was 1 m. The extent of the mysid exclusion zone (MEZ) created by the light was determined by selecting the reports in which at least 70% of the depth bins had S_v values equal to the minimum threshold value (–100 dB). The width of the MEZ was determined by counting the number of reports without mysids detected before and after the light source. Because of the spatial overlap between reports, we added a distance equivalent to half of a report at each end of the MEZ. The distance traveled within the MEZ for each individual transect was then calculated based on the average boat speed, the number of reports, and the distance per report. The values for all transects for a given bulb were averaged to produce the average extent of the MEZ for that particular bulb.

Mysid vertical distribution

Several day and nighttime cruises on Lake Ontario and Cayuga Lake were carried out during the spring, summer, and fall of 1995 and 1996. The Lake Ontario cruises were conducted aboard the RV *Seth Green* over bottom depths of 35–150 m north of Oswego, N.Y. Cayuga Lake cruises were conducted on a small 6-m boat and encompassed most of the lake.

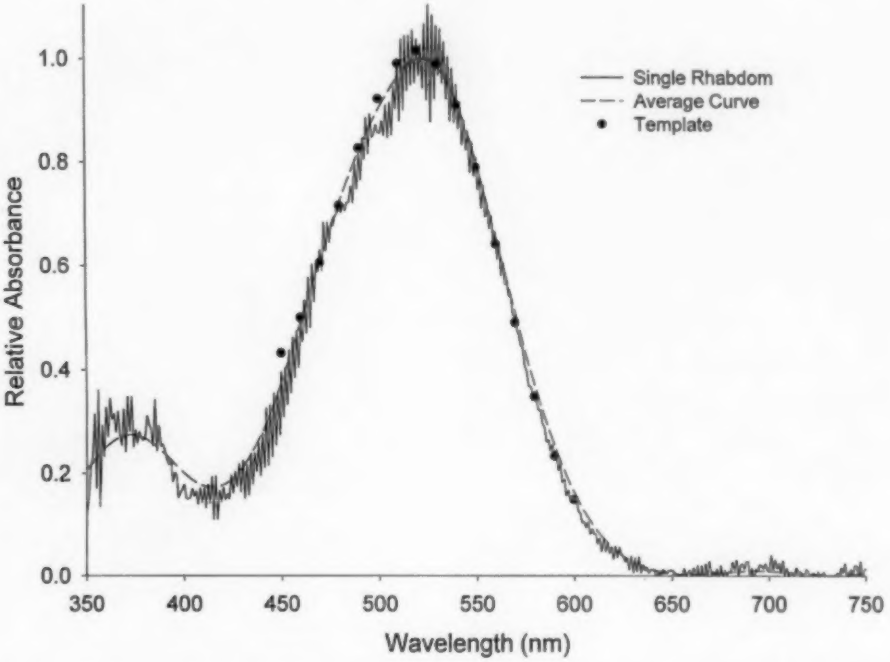
The acoustic system used for the light experiments was also used to determine the mysid vertical distribution (but with a ping rate of 1 $\text{ping}\cdot\text{s}^{-1}$). For both Lake Ontario and Cayuga Lake, segments of acoustic data (50–200 pings long) were selected for analysis based on low noise levels and lack of large individual targets (presumably fish) in the water column above the mysid layer. The mysid layer was identified visually on the echogram and was clearly separable from fish targets. The scattering layer was also identified as mysids based on stratified sampling with a 1-m-diameter conical net (0.5-mm mesh size). The relative density of mysids in the water column was defined by summing the total amount of acoustic backscattering over the entire water column, excluding fish and the top 5 m of the water column, and calculating the relative amount in each depth bin.

Environmental data

Prior to the surveys, we collected environmental data that included temperature and light profiles. In Lake Ontario, during the 1995 cruises, both light and temperature profiles were conducted at a deep station (bottom depth = 90 m). During the 1996 cruises, there were two stations at bottom depths of 60 and 120 m. Light extinction information of the PAR wavelength range (400–700 nm) was measured at approximately 1-m intervals from the surface down to the detection limit with a calibrated LI-193 (Licor, Inc.) underwater spherical quantum sensor. A LI-190 surface quantum cell was used to collect surface irradiance during the profile; this was applied to standardize the profile readings to the amount of available surface light. The vertical extinction coefficient, and, consequentially, the downward irradiance, at a given depth was then calculated using Beer's Law (Kirk 1994). For nighttime calculations of depth-specific light intensity, we applied the calculated extinction coefficients from the daytime profiles to calculated nighttime surface light irradiance. The nighttime irradiance was estimated by adjusting the maximum possible moonlight (clear sky and full moon; see below) to the moon phase and cloud cover for each particular night. Cloud cover information was obtained based on visual observations during the cruises. Temperature profiles were measured using a Hydrolab profiler (depth, temperature, percent oxygen saturation, and pH).

Light and temperature measurements were conducted on Cayuga Lake using the same instrumentation and methods. Temperature and light profiling was conducted prior to sunset at least once on each of the acoustic sampling dates. Thermal structure and light

Fig. 3. Visual pigment of *M. relicta*. The noisy curve is a typical raw data scan from a single isolated rhabdom. The smooth curve is an average of 11 records from the Cayuga Lake sample. The circles are calculated spectral locations for a 520-nm rhodopsin using the Partridge and DeGrip (1991) template data calculated from $y = -588553.45 + 1.45 \times 10^8/x - 1.072 \times 10^{11}/x^2 - 8.413 \times 10^{12}/x^3 + 3.682 \times 10^{16}/x^4 + 6.015 \times 10^{18}/x^5 - 2.487 \times 10^{22}/x^6 + 1.368 \times 10^{25}/x^7 - 3.215 \times 10^{27}/x^8 + 2.894 \times 10^{29}/x^9$ where x is wavelength ($r^2 = 0.999$).



extinction coefficients were calculated and used for analysis of mysid vertical distribution and light experiments.

During the Cayuga Lake daytime cruises (June 12 and 20, July 24, and August 21, 1996), transects progressed from the shallow to the deeper region of the lake until an acoustic mysid layer was clearly identifiable above the bottom. Light profiles were then conducted providing both surface light levels and estimates of water column extinction coefficients (k_{PAR}). In order to apply light extinction values from the profiles (typically <30 m deep) to mysid depths (>60 m), we had to compensate for differences between shallow and deep extinction values. The appropriate k_{PAR} was therefore estimated in two steps. First, we calculated the light intensity at 15 m based on k_{PAR} for the top 15 m of the water column and the range of surface light intensities that we observed. Next, we calculated an average k_{PAR} for the bottom 10 m of each light profile and applied that to the calculated light level at 15 m. Light at the depth of mysid layer was then calculated using Beer's Law. Wavelength-specific extinction coefficients information for the appropriate k_{PAR} were derived from Jerome et al. (1983). In combination with measured surface light levels and sunlight spectral output (similar to moonlight, Munz and McFarland 1973), we estimated light levels in mylux (defined below) at the depth of the mysid layer.

Measurements of the normalized spectral irradiance (λ_N) of the moon were carried out on a clear night (full moon, September 17, 1997) in Ithaca, N.Y., and displayed high irradiance between 545 and 678 nm (Fig. 1). Total available moonlight was taken from the literature (2.08×10^{-4} W·m^{-2}, Morgan and Smith 1981) (value was converted from micromoles of photons per square metre per second following Biggs (1986)). The measurements were combined with the spectroradiometer data ($k_d(\lambda_i)$), the moon phase (M_p, fraction of full moon), and fraction of clear sky (C_c ranging between 0.1 and 1, where 1 represents a clear sky) to provide estimates of wavelength-specific light intensities ($I_z(\lambda_i)$) at a given depth (z) in the following way (Jerome et al. 1983):

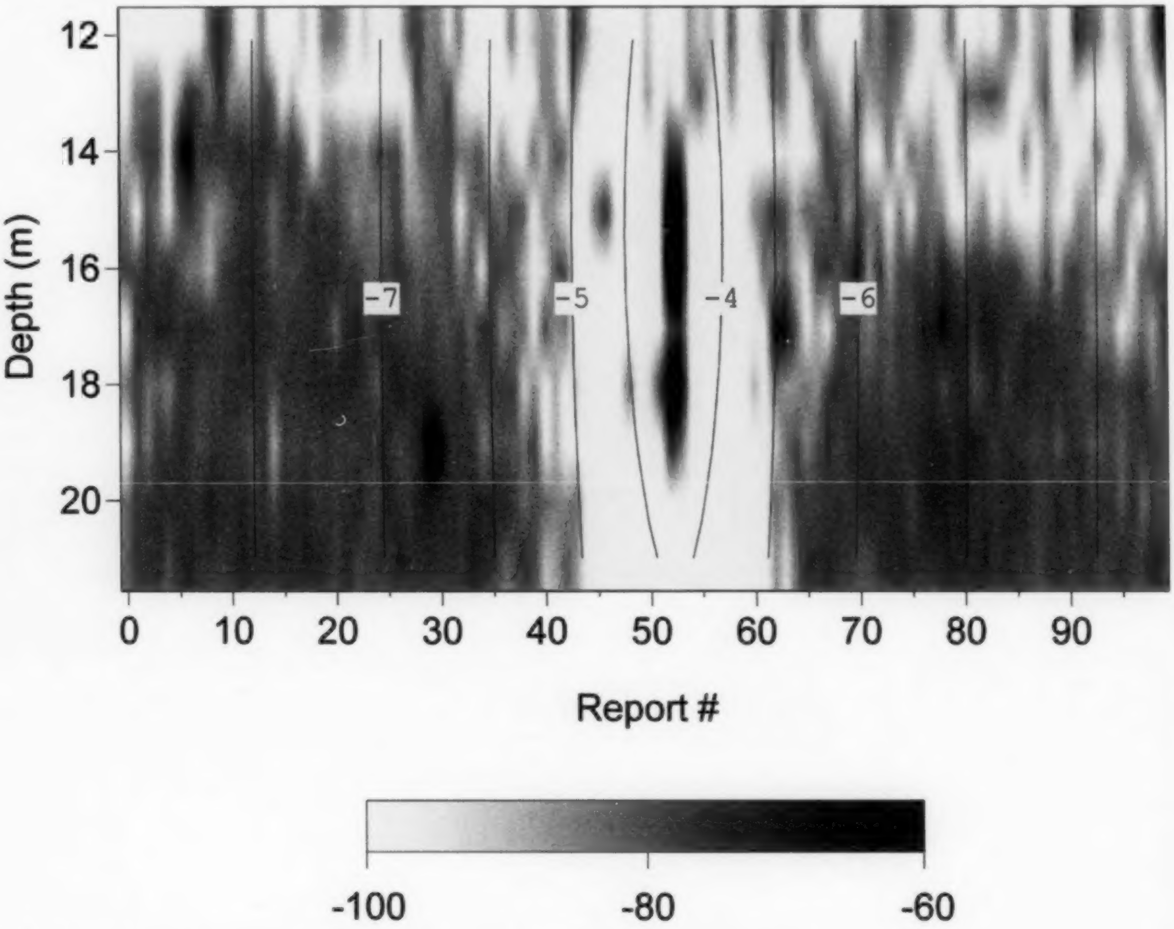
$$(2) \qquad E_d(z, \lambda_i) = E_d(0, \lambda_i) \cdot e^{(-k_d(\lambda_i)z)}$$

where $E_d(z,\lambda_i)$ is the downward irradiance intensity at depth z and wavelength λ_i and

$$(3) \qquad E_d(0, \lambda_i) = 2.08 \times 10^{-4} \cdot M_p \cdot \lambda_{N,i} \cdot C_c.$$

The $k_d(\lambda_i)$ was calculated for 1-nm intervals over the PAR range. We then filtered the light at each wavelength through a scaling procedure that converted the available light to light detected by the mysid eye. This was performed by applying the normalized mysid visual spectrum to estimated light available (watts per square metre) at each depth and wavelength ($E_d(z,\lambda_i) \cdot A_m(\lambda_i)$ where $A_m(\lambda_i)$ is the relative absorbance by mysids at wavelength λ_i). The results were then summed over a wavelength range of 400–650 nm, producing a total amount of light detected by the mysid eye. The scaling is consistent with lux units in that it represents a scaling to the spectral sensitivity of an animal. Unlike lux units that are traceable to a defined light intensity (foot candles or lumens, Billings 1963), the mylux is an application of a wavelength-specific filter to units of watts per square metre. Comparison of light intensity in watts per square metre over the 400–650 nm range with the mylux values for both moonlight and a tungsten bulb light source provided a conversion factor between watts per square metre and mylux. For the remainder of the paper, all light intensities were calculated in mylux and then converted to other units using these conversion factors. For moonlight, 1 mylux = 0.51 W·m^{-2}, 2.35 µmol photons·

Fig. 4. Grayscale-coded distribution of mysids during the course of a transect overlaid with a contour plot of the estimated light levels (mylux units) at varying distances from the light source. The mysid distribution was bilinearly interpolated to smooth the transitions between pixels. Contours are log-transformed light levels and range between –8 and –4. The grayscale bar represents the S_v scale used (–100 to –40 dB). The strong scattering found between about 14 and 19 m deep at report 55 is sound scattered by the light apparatus. The estimated distance per report was 1.05 m.



m^{-2}·s^{-1}, or 175 lx. For the tungsten light source used during the light experiments, 1 mylux = 0.46 W·m^{-2}, 2.12 µmol photons·m^{-2}·s^{-1}, or 157 lx.

Results

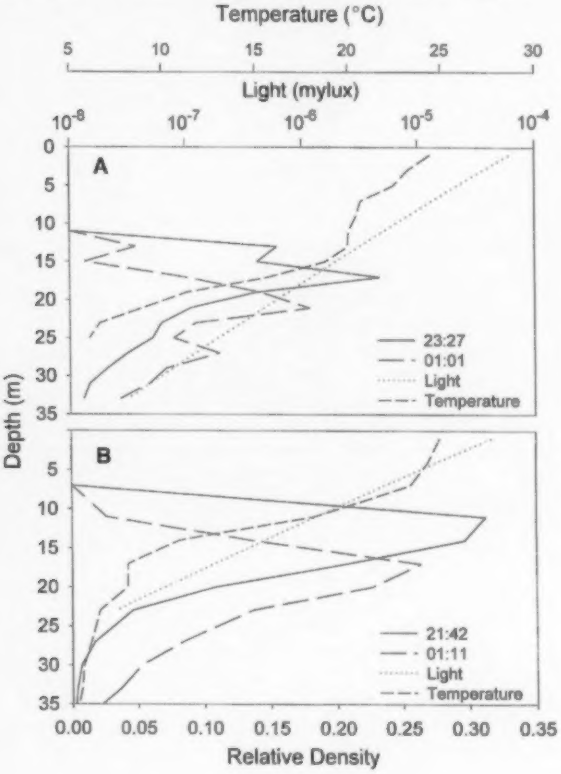
Visual pigment analysis

Only a single visual pigment was found in all rhabdoms examined (from three animals). The results were identical in all cases. The visual pigment has a λ_{max} at 520 nm and is best fit by a vitamin A_1 template curve (Fig. 3). Superimposed on a typical raw data scan (noisy curve) is an average of 11 records from Cayuga Lake mysids. Calculated spectral locations for a 520-nm rhodopsin are also shown. There was no evidence of any additional photopigment. The spectrum displays extremely low sensitivity to light at wavelengths of 620 nm or greater. A second minor peak at 370 nm was observed.

Light experiments

The physical presence of the apparatus used to generate light in the water column at the depth of the mysid layer had no effect on the mysid distribution. There was no clear change in acoustic backscattering detected along the light-off transects. The existence of a light source had a clear effect on the distribution of the mysids in the water column (Fig. 4). The acoustic backscattering (s_v) from the water column was significantly reduced at the depth corresponding to the light source. The light created an MEZ in which mysids were not detectable acoustically. Separate and pooled average s_v values for both bulbs for each region (before MEZ, MEZ, after MEZ) were compared (Wilcoxon signed rank test, $n = 36$) and displayed highly significant differences ($p < 0.005$) between the MEZ s_v values and those of the regions before and after the MEZ for both bulbs. This was not the case when the light was off. The size of the MEZ varied between transects but was on average larger for the bright light source. The mean diameter (SD, range) of the MEZ

Fig. 5. Vertical distribution of *M. relicta* for two time periods during the nights of (A) August 15–16, 1995, and (B) August 17–18, 1995, in Lake Ontario. For each night, two representative mysid vertical distribution profiles are shown. In addition, temperature and estimated light profiles are plotted for each night. Note the differences in the depth of the thermocline and the slope of the estimated light levels between the two nights.



was 20.6 reports (± 2.8, 17–25.5) for the 3.36-W bulb and 24.7 (± 3.9, 19–31) for the 7.54-W bulb. Applying the average distance traveled per report (1.05 m), we estimated the mean (minimum, maximum) MEZ diameter to be 21.6 m (17.8, 26.8) for the 3.36-W bulb and 25.9 m (19.9, 32.5) for the 7.54-W bulb.

The estimated light intensities in mylux units were laid over the mysid distribution for one of the acoustic transects (Fig. 4). For this particular transect, the extent of the MEZ on one side was limited to 7.6×10^{-6} mylux and on the opposite side to 1.1×10^{-5} mylux, both at a depth of 15 m. These values were higher than for most transects. The estimated dimensions of the MEZ were translated to estimated light levels at the perimeters of the MEZ. The mean (minimum, maximum) light intensity at the perimeter of the MEZ was 3.4×10^{-7} mylux (1.1×10^{-7}, 8.4×10^{-7}) for the 3.36-W bulb and 5.8×10^{-7} mylux (1.6×10^{-7}, 2.1×10^{-6}) for the 7.54-W bulb.

Environmental conditions

The light extinction coefficients (k_d) varied both temporally and spatially in Lake Ontario. In general, the nearshore waters of the lake were clearer than the offshore stations, a phenomenon that can be attributed to the abundance of zebra mussels (*Dreissena polymorpha*) in the shallower regions of the lake (Fahnenstiel et al. 1995). Temporally, we found the clearest waters during our May 1996 cruises followed by the October 1995 cruises. The overall range of extinction coefficient values (k_{PAR}) was not large, between 0.22 and $0.4 \cdot m^{-1}$ at a depth of 10 m and between 0.17 and $0.35 \cdot m^{-1}$ at 20–30 m. In general, the August values were higher than during other periods of the year, and the highest values were at the offshore station during the August 17–18, 1995, cruise. Our measurements from Cayuga Lake fell within the Lake Ontario ranges.

The temperature profiles of the water column over the corresponding time period varied between a well-mixed water column (May 1996, range 2.6–3.4°C) and a highly stratified water column in the summer with a thermal range of 24.1–6°C. During the autumn cruise, an erosion of the epilimnion had started, causing a deepening of the thermocline and cooling of the surface waters. This lead to a thermal range of 15–6°C.

Mysid vertical distribution

A total of 16 time periods were examined over six different dates (August 15 and 18 and October 10 and 12, 1995, and May 7 and 8, 1996; example in Fig. 5). We plotted the relative abundance of mysids against calculated light levels in mylux. In none of these 16 distributions did we find more than 10% of the population above 1.9×10^{-6} mylux, which is slightly higher than the value observed to limit the distribution in the light avoidance experiments (3.4×10^{-7} to 5.8×10^{-7} mylux). In May, the mysids are spread over a larger range of light levels (with 90% of the population above 2.25×10^{-9} mylux, Table 1) than in August and October, and the different depth profiles show very similar distribution relative to light levels (Fig. 6). In August, there was a more pronounced threshold effect as evidenced by the similarity in the light levels corresponding to 10 and 90% of the population (Table 1). The distributions of mysids relative to light in August and October were also more variable (Fig. 6). Light levels at depth varied more in August, as there was a relatively large difference in moon phase and extinction coefficient between the two nights investigated (August 15–16, 72% of the moon illuminated; August 17–18, 53% of the moon illuminated). The variability of the vertical mysid profiles related to light levels in August and October suggests that other factors also limit mysid distributions. The average size of mysids caught at the different time periods was similar and ranged between 8 and 13 mm.

In Cayuga Lake, during the four daytime cruises (Table 2), mysids were found between 60 and 70 m with one exception (Aug. 21, 1996) when they were found between 87 and 90 m. The light levels corresponding to those depths varied between sampling dates and ranged between 3.1×10^{-8} and 1.1×10^{-5} mylux.

Discussion

Light sensitivity

The spectral sensitivity of *M. relicta* eyes has been studied previously (e.g., Beeton 1959; Lindström and Meyer-

Table 1. Estimated light levels at a depth representing the shallowest 2.5, 10, and 50% portions of the mysid population for three seasons.

Season	2.5% (mylux)	10% (mylux)	50% (mylux)	90% (mylux)
Spring (May 1996)	4.0×10^{-6}	1.4×10^{-6}	1.4×10^{-7}	2.25×10^{-9}
Summer (Aug. 15, 1995)	3.0×10^{-6}	1.9×10^{-6}	2.9×10^{-7}	2.9×10^{-7}
Summer (Aug. 17, 1995)	1.2×10^{-6}	4.6×10^{-7}	7.6×10^{-8}	1.45×10^{-8}
Fall (Oct. 1995)	1.3×10^{-8}	5.2×10^{-9}	8.9×10^{-10}	1.8×10^{-11}

Note: Light levels represent the light intensities at the depth at which at least 2.5, 10, 50, and 90% of the population was found. Similar light intensities at the 10 and 90% levels would indicate a highly aggregated and stratified mysid distribution. See text for mylux calculation. Light levels in µmol photons·m^{-2}·s^{-1} and lx units can be calculated using the conversation factors in the text.

Rochow 1987; Lindström et al. 1988), but not by direct observation of the pigment absorbance as in this study. We found a single visual pigment with the characteristics of vitamin A_1 based rhodopsin and a peak spectral sensitivity of 520 nm for Cayuga Lake mysids. Beeton (1959) determined a peak sensitivity at 515 nm for mysids from Lakes Michigan and Huron. Herman (1963) studied the marine mysis *Neomysis americana* using methodology identical to Beeton's (1959) and found a similar peak sensitivity at 515 nm. Both Beeton's (1959) and Herman's (1963) observations can be considered identical to ours because they used light through discrete filters (395, 430, 485, 488, 496, 515, 540, 610, and 640 nm) to measure mysid reaction time to the light. The closest wavelength to the 520-nm peak sensitivity observed by us is 515 nm. Beeton (1959) also reported an additional peak reaction time at 395 nm and suggested that *M. relicta* possesses at least two visual pigments. The secondary peak is close in location to a secondary peak in that we identified in our study (370 nm). This secondary peak most likely does not represent an additional pigment but rather is a result of one of two possibilities: (*i*) it represents a photoproduct that can both absorb and stimulate (Menzel 1979) or (*ii*) it is a β band of the pigment that is at the same location as the photoproduct. The β band is always at a shorter wavelength than the main absorbance band (α) and is capable of stimulating vision (Warrant and Nilsson 1998).

Lindström and Meyer-Rochow (1987) and Lindström et al. (1988), recording electroretinograms, found peaks at 505, 550, and 570 nm from three different locations in Finland and the Baltic Sea. They suggested that the differences in peak sensitivities between populations resulted from deviation in the optical characteristics and the photic conditions of the water bodies in question. In their studies, the mysids' peak sensitivities (505, 550, and 570 nm) at the sites that they examined corresponded to peak irradiances of 555–575, 565–585, and 630–690 nm, respectively, showing that the peak sensitivity of *M. relicta* was consistently lower than the peak irradiance (between 25 and 90 nm). In Cayuga Lake the peak sensitivity of 520 nm was 43 nm lower than our measurements of peak irradiance (563 nm at a depth of 20–23 m). Similarly, the peak sensitivity of Lake Huron mysids (515 nm) measured by Beeton (1959) was 35 nm lower than the peak irradiance reported by Jerome et al. (1983). Thus, available data to date indicate that *M. relicta* is most sensitive to light between 505 and 570 nm and that the peak sensitivity varies between populations in response to differences in the optical environment.

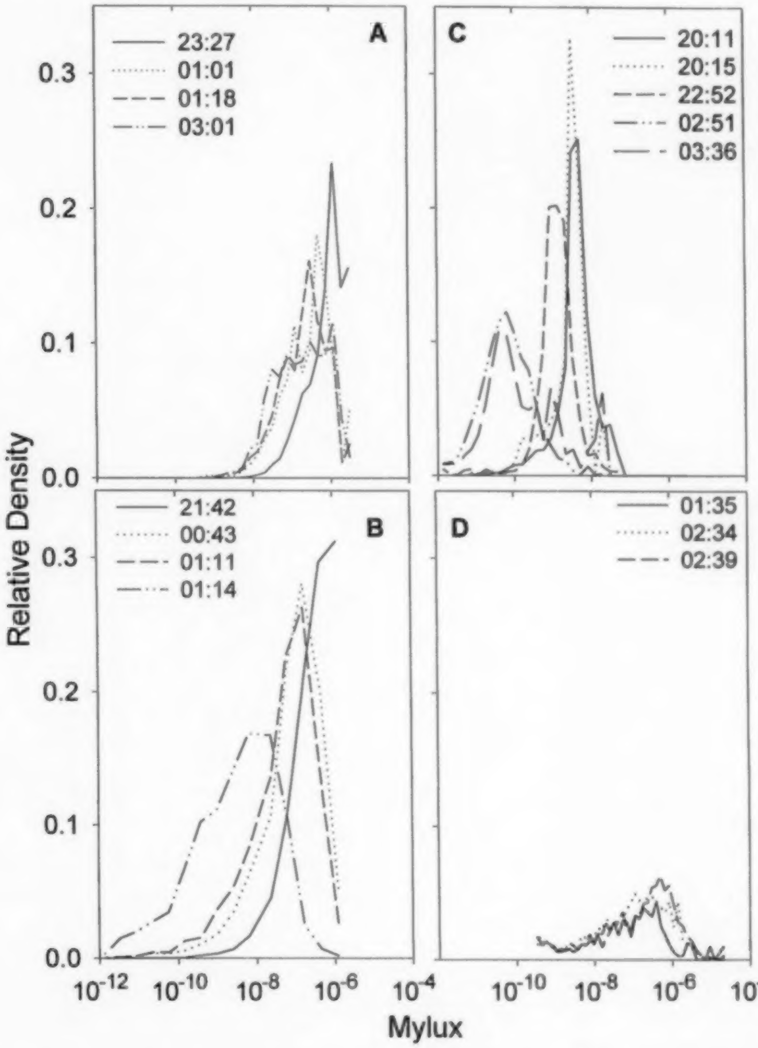
Past studies of crustacean visual spectral sensitivity have focused on marine species (e.g., Frank and Case 1988). Most deepwater species investigated (daytime residence at a depth of several hundred metres) displayed maximal spectral sensitivity close to 500 nm and possessed a single visual pigment, although some deepwater species were found to have an additional peak sensitivity in the UV range and a visual system containing two visual pigments (Frank and Widder 1994). It has long been assumed that the visual systems of deep-sea organisms are adapted for maximum sensitivity to the ambient light spectra (blue range), and this has been shown to be the case for many species (Frank and Case 1988 and references therein). However, our results for *M. relicta* show an offset between the environmental peak irradiance and the organism's peak sensitivity, thereby supporting the "contrast" hypothesis. This hypothesis states (see complete discussion in Loew 1995) that the pigments are positioned to exploit the difference between the spectral distribution of the background spacelight and the spectral reflectance of the targets causing the peak irradiance and peak sensitivity not to overlap.

As in the case of *M. relicta*, the deepwater marine crustaceans are highly sensitive to very low light levels (Frank and Widder 1994). Sensitivity to low light levels would allow them to search for prey under reduced predation pressure and increased success of prey capture. Ramcharan and Sprules (1986) showed increased prey capture rate under dim light conditions over complete darkness.

Measuring light levels relevant to mysid perception: the mylux unit

Light intensity measurements described in terms of photometric units refer to light measurements of visible radiation using a sensor with the spectral characteristics equal to those of the human eye. We adapted the same approach by correcting radiant light flux to radiation that is measurable by a sensor equal to that of a mysid eye. This is the correct measure of light levels perceived by the mysid but requires either a light sensor with a spectral response identical to that of mysids or additional environmental information (e.g., wavelength-specific extinction coefficients, spectral output of light source, etc.). Whether measurements in the mylux scale are needed depends on the spectral characteristics of the available light. The ambient light on a typical cloudless night (Fig. 1) displays peak relative intensities at the longer wavelengths (about 590 –670 nm). The light source used for the light experiments had peak intensities shifted further to the red (about 620–730 nm). In both cases, a significant portion of that light is not perceived by the mysids. The optical characteristics of the water column during this study (Fig. 2 and Jerome et al. 1983), by absorbing the long-wavelength

Fig. 6. Relative distribution of mysids as a function of light for three seasons. (A) August 15–16, 1995; (B) August 17–18, 1995; (C) October 10–11, 1995; (D) May 7, 1996. Estimated available light calculated from moonlight normalized spectra, spectroradiometeric data, and the mysid normalized eye spectra (see text for details of mylux units). Each seasonal distribution consists of three to five different time periods over the course of a night. Each curve represents the relative density of mysids as a function of light.



(>600 nm) light unavailable to mysids, provided some correction to the incompatibilities of the light sources and mysid spectral sensitivity.

Light levels measured at different depths depend on the sensor used. For example, mysids are predicted to move up from 13.5 m (in full moonlight with a k_{PAR} of $0.3 \cdot m^{-1}$) to 4 m with 15% of the moon illuminated when distributions are predicted using equivalent mylux units. With other units, the depth change would be from 17.5 to 2 m (using micromoles of photons per square metre per second), from 16.5 to 1 m (using lux), and from 18.5 to 9 m (using watts per square metre) The comparison illustrates the importance of mylux, not only in predicting relative changes in the vertical distribution of mysids but also in predicting the absolute depth distribution in the water column. The magnitude of the differences depends greatly on the spectral properties of the water and the organism. In cases of significant differences between the sensor's spectral sensitivity and the spectral characteristic of the water column such as in lakes rich in dissolved organic compounds, the use of units or sensors representative of the organism's vision is critical.

Evidence of light threshold

Both the results of the light experiments and the observations on nighttime distributions indicate a light threshold for mysids in Lakes Cayuga and Ontario of about 3.4×10^{-7} to 2.1×10^{-6} mylux. The size of the MEZ was larger for the brighter bulb. The mysids were found at distances corre-

Table 2. Mysid daytime distribution and light levels. All observations are from Cayuga Lake and from 1996.

Date	Mysid layer depth (m)	k_{PAR} Shallow	k_{PAR} Deep	Light at mysid layer (mylux)
June 12	59–70	0.41	0.37	3.1×10^{-8} to 8.1×10^{-7}
June 20	65–68	0.40	0.26	6.7×10^{-6} to 1.1×10^{-5}
July 24	61–70	0.44	0.28	6.1×10^{-7} to 5.2×10^{-6}
Aug. 21	87–90	0.42	0.22	4.1×10^{-8} to 8.5×10^{-8}

Note: Due to technical failure, only one surface reading for the June 20 profile was available. The estimated light range at the depth of the mysid layer accounts for the minimum and maximum light intensities at the surface while the light profiles were conducted and the range of depths of the mysid layer (shallowest portion of the mysid layer) over the course of the observations. Estimated light levels in µmol photons·m^{-2}·s^{-1} and lx units can be calculated using the conversion factors in the text. The wavelength-specific extinction coefficient values were calculated based on the k_{PAR} value and extrapolated from the curve in Jerome et al. (1983) (see text). The light levels at mysid depth were calculated in two steps. First, we calculated the light intensity at 15 m based on k_{PAR} for the top 15 m of the water column and the range of surface light intensities that we observed. Second, we calculated an average k_{PAR} for the bottom 10 m of each light profile (eq. 3). The "deep" k_{PAR} was then applied to estimated light level at 15 m and a depth range corresponding to the mysid depth (minus 15 m). The applied k_{PAR} values are provided.

sponding to mean light levels of 3.4×10^{-7} to 2.1×10^{-6} mylux for both bulbs. The consistency of the estimated light levels at the edge of the MEZ for both the 3.36- and 7.54-W bulbs supports these threshold values. The MEZ was caused by the animals avoiding the higher light levels and not by the changes in orientation in response to light (which would cause lower acoustic returns). Only a few mysids were caught with nets within the MEZ (0.6 mysid·m^{-2}) as opposed to when the light was off (30.7 mysids·m^{-2}). In contrast with the results presented by Teraguchi et al. (1975) for their fall light experiments, we did not observe mysids returning to their original distribution while the light was on, regardless of the length of time involved.

The same light threshold limited the vertical distributions observed at night in Lake Ontario. In all depth profiles investigated, 90% of the mysid population was always below the 2×10^{-6} mylux threshold (Fig. 6). The spring mysid distribution was spread relatively evenly over a wide range of light intensities (4.0×10^{-6} to 2.25×10^{-9} mylux), with a few animals up to as bright as 1.2×10^{-5} mylux (<1% of the population). The distribution coincided with a well-mixed water column; therefore, avoidance of high temperatures would not have limited upward vertical migration. In August, the mysids displayed a clear "threshold" distribution characterized by a sharp increase in mysid density just below a certain light threshold value, thereby creating a very dense and well-defined scattering layer during the night. However, the light levels corresponding to this threshold in distribution only occasionally matched the light threshold observed in the light experiments (Fig. 6). This is likely due to the thermal structure. For example, during the brighter August night, the top 10% of the mysid population was found in higher light levels (1.9×10^{-6} mylux) than during the darker night (4.6×10^{-7} mylux) because temperature restricted their movement to the brighter light levels during the darker night (Fig. 6). In order for the mysids during the darker night (Aug. 17–18) to be at 2×10^{-6} mylux, they would have had to reside at a depth of about 9–10 m. This would have placed them in water of 20–21°C, a temperature potentially lethal to mysids (DeGraeve and Reynolds 1975). In October, mysids were found deepest in terms of overall light intensity (Fig. 6) with a threshold distribution at light levels of 1.3×10^{-8} mylux. This distribution in low light is consistent among the different profiles. The thermocline had eroded in October and was generally found between 17 and 35 m, which was shallower than the mysid distribution. However, depth of the thermocline was quite variable during the survey and could have restricted mysid migration. An alternative explanation for distribution of mysids in darker waters in October is the presence of higher densities of rainbow smelt (*Osmerus mordax*) and alewife (*Alosa psuedoharengus*) that feed on mysids (Mills et al. 1992 and personal observation) in the water column.

The light threshold that we observed (1.0×10^{-6} mylux or 1.7×10^{-4} lx) was lower than observations made by Teraguchi et al. (1975). They found the leading edge of the ascending mysid layer to be associated with light levels of about 0.3–0.03 lx. The discrepancy between their observations and ours may stem from differences in the methods used to measure light. Unfortunately, there is insufficient information in their study to directly compare their observations with ours. Our threshold light levels are similar to reported thresholds for *M. mixta* in the Baltic Sea (Rudstam et al. 1989). Ideally, comparisons should be made with a unit of light level related to the organism's spectral sensitivity in a given lake.

The light level at the top of the mysid scattering layer during the day in Cayuga Lake ranged between 3.1×10^{-8} and 1.1×10^{-5} mylux on four different occasions (June 12 and 20, July 24, and August 21, 1996, Table 2). These values encompass the light threshold obtained from the light experiments and the night distributions. Extinction coefficients used for calculating daytime light levels had to be extrapolated from measurements in 20- to 30-m-deep water, as there was not enough light to measure extinction at deeper depths with our light meter. This will increase uncertainty in calculated light levels in deep water. Thus, our current data do not refute the hypothesis that mysids avoid the same light levels of about 1×10^{-6} mylux both day and night. Mysids will occur in the water column during the day if the light levels are low enough in Cayuga Lake. Mysids are also found in the open water during the day in Lake Ontario (at depths of 140 m, L.G. Rudstam and O. Johannsson, personal observation).

Does the mysid threshold value coincide with the lowest light levels that allow visual predation by planktivores? Observed light thresholds for fish feeding range between 10^{-2} and 10^{-5} lx for pike, minnow, coho salmon, and carp (Blaxter 1975; Dabrowski and Jewson 1984). Studies have also shown photobehavioral response as low as 10^{-4} lx in catfish (Tabata and Minh-Nyo 1989). The reactive distance of several planktivorous fish (bluegill, crappie, grayling, and lake trout) to 2-mm daphnia has been found to decrease sharply once light levels fall below 1 lx (about 0.018 µmol photons·m^{-2}·s^{-1}) and in some cases 10 lx (O'Brien 1979). While mysids are larger than 2 mm, the daphnia example does illustrate the light limitations imposed on planktivorous fish. The size of prey selected by planktivores is dependent on light intensity. As the intensity of the available light decreases, predators select for increasingly larger prey (O'Brien 1979; Mills et al. 1986). We have not converted the values to mylux due to lack of wavelength-specific information for the light sources used in the experiments cited here. Assuming a source with a spectrum identical to moonlight, a light intensity of 10^{-6} mylux corresponds to 1.75×10^{-4} lx. Thus, it appears that the mysid light threshold coincides with the lowest light levels for visual feeding by fish. But the lack of information on the visual system of mysids' direct predators (alewife and smelt) prevents us from concluding whether the light threshold overlaps with their predators' light sensitivity threshold in Lakes Ontario and Cayuga. Alewife may feed in total darkness, but only by filter feeding, a feeding mode that reduces its effectiveness in capturing mysids (Janssen and Brandt 1979). Smelt are known to feed on mysids during the night (Brandt and Madon 1986), suggesting that part of the mysid population is at a depth illuminated sufficiently for smelt predation.

There are two important implications of our study. First, we have presented data on the spectral sensitivity of *M. relicta* showing these animals to have one visual pigment with a peak sensitivity of 520 nm. This information was used to scale irradiance at depth to the light perceived by the animals (defined as mylux). Light levels limiting mysid distribution during vertical migration and in field experiments were more similar when measured in mylux units than when measured with standard sensors. It is important to relate light levels in the field to the perception of the animals, especially when comparing across systems. Second, our data support the hypothesis that a threshold light level limits the distribution of *M. relicta* in the water column both day and night. This light level is lower than previously reported for *M. relicta*. However, other factors (temperature, possible predator distributions) will at times limit mysids to lower light levels than this threshold. *Mysis relicta* is an important predator on zooplankton and prey of zooplanktivorous fish in many lakes. Understanding factors limiting its distribution is a prerequisite for understanding these three-way predator–prey interactions.

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Spatial and temporal differences in the diet of Great Lakes herring gulls (*Larus argentatus*): evidence from stable isotope analysis

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Abstract: Stable nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) isotope values were measured in lipid-free homogenates of herring gull (*Larus argentatus*) eggs collected from the Laurentian Great Lakes during 1974–1995. $\delta^{15}N$ values provided an indication of adult gull trophic position during egg formation. After adjustment of egg $\delta^{15}N$ values for interlake differences in baseline food web $\delta^{15}N$ signatures, significant differences in gull trophic position were found among colonies. These results were attributed to differing proportions of fish in the diets of gulls from the various colonies. Aquatic foods available to gulls had greater $\delta^{15}N$ values than terrestrial foods. $\delta^{13}C$ measurements provided some support for our hypothesis that terrestrial foods, particularly garbage, were more enriched in ^{13}C than aquatic foods. As the proportion of fish in the diet decreased, the fraction consisting of terrestrial food increased, resulting in an increase in $\delta^{13}C$ values and a decline in $\delta^{15}N$ values. Stable isotope values for gull eggs from Lake Erie changed significantly during the study period and may have reflected a decline in fish availability.

Résumé : On a mesuré les valeurs des isotopes stables de l'azote ($\delta^{15}N$) et du carbone ($\delta^{13}C$) dans des homogénats sans lipide d'oeufs de goéland argenté (*Larus argentatus*) recueillis dans la région laurentienne des Grands Lacs durant la période 1974–1995. Les valeurs de $\delta^{15}N$ ont fourni une indication de la position trophique des goélands durant la formation des oeufs. Après avoir corrigé les valeurs de $\delta^{15}N$ des oeufs pour tenir compte des différences dans les signatures de fond de $\delta^{15}N$ dans les réseaux alimentaires d'un lac à l'autre, on a observé des différences significatives dans la position trophique des goélands entre colonies. Ces résultats ont été attribués aux proportions différentes de poisson dans les régimes alimentaires des goélands des diverses colonies. Les valeurs de $\delta^{15}N$ des aliments aquatiques que peuvent consommer les goélands sont supérieures à celles de leurs aliments terrestres. Les mesures de $\delta^{13}C$ sont venues appuyer notre hypothèse que les aliments terrestres, particulièrement les déchets, renfermaient plus de ^{13}C que les aliments aquatiques. Quand la part de poisson dans le régime alimentaire diminuait, la part d'aliments terrestres augmentait, d'où un accroissement de $\delta^{13}C$ et une diminution de $\delta^{15}N$. Les valeurs des isotopes stables pour les oeufs de goéland du lac Érié ont changé significativement durant la période de l'étude et peuvent avoir reflété une baisse de la disponibilité du poisson.

[Traduit par la Rédaction]

Introduction

The herring gull (*Larus argentatus*) is a colonially breeding bird distributed throughout north-temperate latitudes. Since the early 1970's, herring gulls from the Laurentian Great Lakes have been used to monitor trends in levels and effects of organochlorine contaminants (Mineau et al. 1984). Recently, the scope of this monitoring program has been broadened to evaluate the utility of the herring gull as an integrator of other ecosystem stressors, including changes in fish availability (Shutt and Hebert 1996). A number of factors, including the nutritional quality of their diet, may affect the physiological condition and reproductive success of herring gulls. Herring gulls feed opportunistically on a variety of aquatic and terrestrial food types including fish, garbage, songbirds, small mammals, amphibians, invertebrates, and vegetation (Fox et al. 1990; Ewins et al. 1994). Previous dietary studies conducted on the Great Lakes used pellets, boli, and faeces as indicators of the herring gull diet (Fox et al. 1990; Ewins et al. 1994). Pellets consist of indigestible food items regurgitated by gulls after feeding and boli are composed of undigested food regurgitated prior to or during capture. In these studies, it was found that during both the breeding season and winter, the primary food consumed by herring gulls was fish, although there was variation among colonies and years. However, the degree to which pellets and faeces accurately reflect total diet composition is uncertain. Easily digested food items such as soft-bodied fish species,

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e.g., alewife (*Alosa pseudoharengus*), may be underrepresented (Duffy and Jackson 1986). Analysis and interpretation of stable isotope values in herring gull tissues can overcome this limitation, provide additional insights into the composition of gull diets, and corroborate the results from these earlier studies (Hobson 1987; Hobson et al. 1994).

Naturally occurring stable isotopes have been measured to examine energy flow through food webs (Peterson and Fry 1987). Stable nitrogen isotope ratios ($^{15}N/^{14}N$), expressed as $\delta^{15}N$ (see below), can provide information on food web interactions and indicate the trophic positions of constituent species (Minagawa and Wada 1984; Hobson and Welch 1992; Cabana and Rasmussen 1994). During amination and deamination of proteins, the heavy ^{15}N isotope is enriched relative to the lighter ^{14}N isotope; this process results in a stepwise increase in ^{15}N with trophic level (Minagawa and Wada 1984). Typically, $\delta^{15}N$ values increase by 3–4‰ from one trophic level to the next (Peterson and Fry 1987). This has also been found to be true for avian eggs, where egg protein $\delta^{15}N$ values were enriched by 3.4‰ relative to the laying female's diet (Hobson 1995).

In single-system studies, several authors have successfully used $\delta^{15}N$ measurements in components of aquatic food webs to delineate trophic relationships (Hobson and Welch 1992). However, $\delta^{15}N$ values associated with primary producers in the Great Lakes may vary geographically or according to nutrient regimes (Wada and Hattori 1978; Owens 1987; Cabana and Rasmussen 1996). Because $\delta^{15}N$ values for primary producers may differ between systems and within systems through time, it may be inappropriate to use $\delta^{15}N$ measurements as an absolute means of determining trophic relationships across systems (Cabana and Rasmussen 1996; Vander Zanden et al. 1997). In cases where food webs differ inherently in $\delta^{15}N$ values of primary producers, it is necessary to correct trophic level estimates using measurements of lower trophic level organisms (e.g., unionid mussels; Cabana and Rasmussen 1994) or through estimates of the relative influence of anthropogenic nitrogen inputs to aquatic systems (Cabana and Rasmussen 1996). The Great Lakes differ significantly in levels of primary productivity and potential input of human sewage and other anthropogenic sources of nitrogen (Neilson et al. 1995). As a result, it is not appropriate to directly compare $\delta^{15}N$ measurements of top consumers, such as herring gulls, without taking into account fundamental differences in $\delta^{15}N$ values among lakes. Since no previous systematic survey of isotope values in the same species among the Great Lakes existed, one goal of this study was to determine whether significant interlake differences exist in baseline isotopic signatures and to use such differences to calibrate our $\delta^{15}N$ values for comparative purposes.

Unlike $\delta^{15}N$, $\delta^{13}C$ shows little consistent trophic enrichment (Peterson and Fry 1987). However, because ^{13}C and ^{12}C react at different rates in biogeochemical reactions, there are large differences in $\delta^{13}C$ signatures among major carbon sources. These differences have been used to determine the origin of carbon inputs into food webs (Rau 1980; France 1997). For example, the $\delta^{13}C$ signature of primary producers in terrestrial and lake ecosystems has been shown to differ (Rau 1980). In general, terrestrial C-3 foliage has a $\delta^{13}C$ value of –28‰ (reviewed in France 1995*a*), whereas lentic phytoplankton exhibit more negative $\delta^{13}C$ signatures, with mean values ranging from –32 to –46‰ (Rau 1980; France 1997). In large lakes, most autochthonous primary production is the form of phytoplankton (Gaedke et al. 1996). Stable carbon isotope values are also potentially useful for discriminating between inshore, or benthically linked, food webs and offshore, or more pelagic, food webs within lakes (reviewed by France 1995*b*).

Since the Canadian Wildlife Service's Herring Gull Monitoring Program began in 1974, egg samples have been collected annually and archived in a specimen bank at the National Wildlife Research Centre, Hull, Que. These egg samples provided a unique opportunity to examine both recent and historic tissues. In this paper, we report on geographic differences and temporal changes in the stable nitrogen and stable carbon isotope composition of archived Great Lakes herring gull eggs and examine the processes that may be responsible for these patterns.

Methods

Herring gull egg collection

Thirteen herring gull eggs were collected annually from each of 13 Great Lakes colonies (Fig. 1). Colonies sampled were Granite Island and Agawa Rocks (western Lake Superior, eastern Lake Superior); Gull Island and and Big Sister Island (northern Lake Michigan, Green Bay); Double Island, Chantry Island, and Channel/Shelter Island (North Channel, Lake Huron, Saginaw Bay); Fighting Island (Detroit River); Middle Island and Port Colborne (western Lake Erie, eastern Lake Erie); Niagara River; and Toronto Harbour and Snake Island (western Lake Ontario, eastern Lake Ontario).

Herring gull pellet collection

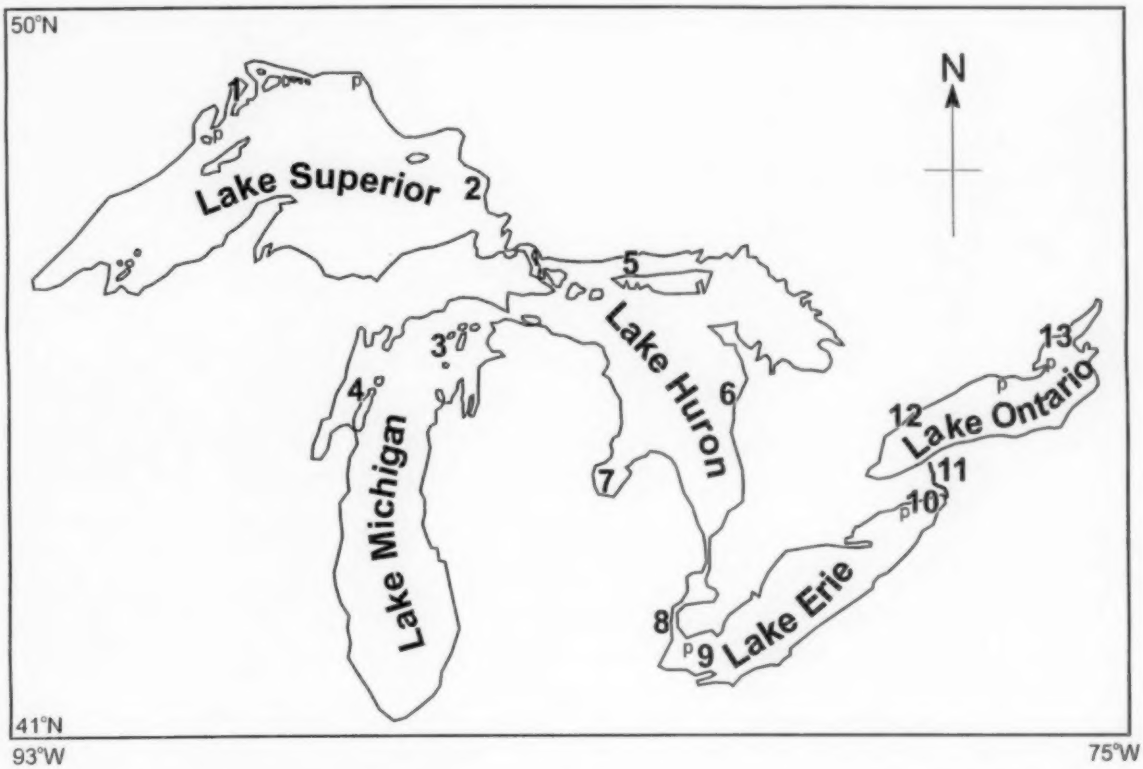
As part of a related study in which the effect of diet composition on adult herring gull physiological condition and reproductive success was examined, pellets were collected from nests on Lakes Superior, Erie, and Ontario in 1995. Pellets were collected from two colonies on each of these lakes. All pellets found within a 1-m radius of individual herring gull nests were collected. These pellets provided evidence of diet composition and were used for comparative purposes with previous studies (Fox et al. 1990; Ewins et al. 1994). Pellets from each lake were individually dissected and the occurrence of the major food types (fish, garbage, songbirds, small mammals, invertebrates, and vegetation) in all pellets from each lake was calculated as a percentage. A *G* test with a Williams correction (Sokal and Rohlf 1981) was used to test for statistical differences in the composition of herring gull pellets from the lakes.

Colonies from which pellets were collected were not the same as those from which eggs were collected (see Fig. 1). Lake Superior pellets originated from a colony near the Granite Island colony (Silver Islet) and another colony in the central part of the lake (Marathon). Lake Erie pellets originated from colonies in the western (Middle Sister Island near Middle Island) and eastern (Mohawk Island near Port Colborne) basins. Lake Ontario pellets were collected in the central/eastern part of the lake (Scotch Bonnet Island and False Duck Island near Snake Island). Although these pellets did not originate from the identical colonies from which eggs had been collected, they do provide a general indication of interlake differences in diet composition.

Stable isotope analysis of herring gull eggs

In general, individual homogenates of the 13 eggs from each colony were pooled by colony and year on an equal weight basis;

Fig. 1. Herring gull colonies from which eggs were collected. 1, Granite Island; 2, Agawa Rocks; 3, Gull Island; 4, Big Sister Island (Green Bay); 5, Double Island; 6, Chantry Island; 7, Channel/Shelter Island (Saginaw Bay); 8, Fighting Island; 9, Middle Island; 10, Port Colborne; 11, Niagara River; 12, Toronto Harbour; 13, Snake Island. Locations of the colonies where herring gull pellets were collected are indicated by the letter p.



therefore, in most cases, only one combined sample was analysed per colony per year. However, the 13 egg samples collected annually from the two Lake Erie colonies from 1990 to 1995 were analysed individually. Individual analyses were conducted to determine the degree of variation within a colony for specific years and were only completed for samples from Lake Erie because of economic considerations. Samples from Lake Erie were chosen for individual analysis because of the ecological changes that have occurred in that ecosystem in recent years (e.g., introduction of dreissenid mussels; Leach 1993; Nicholls and Hopkins 1993; Howell et al. 1996). When isotopic results from individual samples were available, a mean stable isotope value was calculated and this mean value was used in the examination of geographic and temporal trends. The homogenates and individual samples were stored at –40°C until analysed. Further details regarding sample collection, storage, and processing have been reported previously (Mineau et al. 1984).

From most colonies, only samples collected from 1982 to 1995 were analysed. Data are not available for nine of the 182 colony–year combinations during this period (Granite Island, 1983; Gull Island, 1986; Big Sister Island, 1982, 1984, 1992; Channel/Shelter Island, 1982; Port Colborne, 1984 and 1994; Niagara River, 1990). Analysis of egg samples collected before 1982 was only completed for samples from Granite Island (1974, 1976, 1978, and 1980–1981), Agawa Rocks (1978–1981), and Snake Island (1977–1981).

One-millilitre subsamples of the pooled herring gull egg homogenates or individual eggs were freeze-dried and lipids were removed using several 2:1 chloroform–methanol rinses. Stable nitrogen and stable carbon isotope analyses were conducted on these lipid-free subsamples. For $\delta^{15}N$ and $\delta^{13}C$ analysis, about 1 mg of powdered sample was weighed into tin cups and combusted at 1800°C in a Robo Prep elemental analyser interfaced with a Europa 20:20 continuous-flow isotope ratio mass spectrometer. Samples were placed in a sequence of two laboratory standards (albumen) for every five unknowns. Stable nitrogen and stable carbon isotope ratios were expressed in delta (δ) notation, as parts per thousand deviation from a standard. Nitrogen and carbon stable isotope standards were atmospheric nitrogen and Pee Dee Belemnite limestone, respectively. Based upon hundreds of replicate measurements of laboratory standards, we estimate analytical precision to be ±0.1‰ for carbon and ±0.3‰ for nitrogen.

Geographic differences in egg isotopic signatures

Adjusting for interlake differences in baseline $\delta^{15}N$ signatures

To examine potential interlake differences in baseline isotope signatures, rainbow smelt (*Osmerus mordax*) were collected from all of the Great Lakes during 1997. Smelt were chosen because they are found in all study lakes and represent one of the most important prey fish species in the Great Lakes. After collection, fish were frozen until processed. Only adult fish 110–120 mm total length were utilized. Whole, individual smelt were homogenized and five individuals were pooled into each sample. For each lake, five pooled samples representing 25 fish were analysed. These

Table 1. Main constituents of herring gull pellets collected from lakes Superior, Erie, and Ontario in 1995.

		Main pellet constituent				
Lake	Total *n*	Fish	Bird	Plant	Garbage	Other
Superior	127	9	13	4	91	10
% of total		7.1	10.2	3.1	71.7	7.9
Erie	49	26	5	4	12	2
% of total		53.1	10.2	8.2	24.5	4
Ontario	17	8	1	2	6	0
% of total		47	5.9	11.8	35.3	0

pools were freeze-dried and underwent lipid extraction prior to stable isotope analysis. Isotope analysis was conducted in an identical manner as for the egg samples.

Significant interlake differences in $\delta^{15}N$ (Kruskal–Wallis, $H = 22.9$, $p < 0.001$) and $\delta^{13}C$ (Kruskal–Wallis, $H = 15.7$, $p < 0.01$) in smelt samples (see Table 4 for values) indicated that it was necessary to adjust herring gull $\delta^{15}N$ values for these differences to allow for meaningful interlake comparisons. This adjustment was ultimately accomplished by transforming the gull $\delta^{15}N$ values into an estimate of gull trophic position (TP) using a modified version of Cabana and Rasmussen's (1996) equation:

$$TP = [(\delta^{15}N_{gull} - \delta^{15}N_{fish})/3.4] + 3.$$

This modified equation accounted for the fact that we used secondary consumers, i.e., prey fish (trophic level 3), as a means to account for baseline differences in lake $\delta^{15}N$ signatures. Based upon results reported by Vander Zanden and Rasmussen (1996), the assumption that prey fish occupied trophic level 3 may have been an underestimate. Therefore, gull trophic positions could be slightly greater than estimated.

An important consideration that had to be addressed prior to using this equation was to accurately assess the isotopic signature of the gulls' prey fish in each lake. We could not simply adjust the herring gull data using the smelt results because smelt is not the major prey fish species that is consumed by herring gulls in all the Great Lakes. For each lake, we estimated the major prey fish species that would likely be consumed at colonies on that lake. For Lakes Ontario and Erie, the selection of the appropriate fish species was based, in part, upon previously published herring gull dietary studies (Fox et al. 1990; Ewins et al. 1994). Unfortunately, data of this type do not exist for all the Great Lakes. Therefore, for all the lakes, estimates of the relative abundances of the major prey fish species (Schaner and Schneider 1996; Bronte and Hoff 1997; Lake Erie Forage Task Group 1997; Madenjian et al. 1997; Curtis et al. 1998) were also considered under the assumption that the most abundant fish species would also be those most frequently consumed by gulls. Another factor that we considered was that not all prey fish species are equally vulnerable to predation by gulls. Spatial segregation of fish predators and their potential prey may occur as a result of differences in thermal preferences (Goyke and Brandt 1993). This spatial separation of predator and prey is even more important for birds feeding on fish at the water's surface. Utilization of prey fish species by gulls will depend upon the degree to which specific fish species frequent the surface waters of the lake, thereby making them vulnerable to gull predation. For each lake, egg $\delta^{15}N$ signatures were adjusted using the following species: Lake Superior, rainbow smelt and lake herring (*Coregonus artedi*); Lake Huron, rainbow smelt; Lake Michigan, alewife; Lake Erie, emerald shiner (*Notropis atherinoides*), freshwater drum (*Aplodinotus grunniens*), gizzard shad (*Dorosoma cepedianum*), rainbow smelt, white perch (*Morone americana*), and yellow perch (*Perca flavescens*); Lake Ontario, alewife. Isotope data for these species were obtained from published sources (Kiriluk et al. 1995; Keough et al. 1996) as well as from the analysis of samples completed as part of this study. All of the fish samples analysed in this study were collected in 1997 and were treated in an identical manner to the smelt samples discussed above. For each species, analyses were conducted on three pools, with each pool containing three fish. Mean total length and mass of the fish analysed were also recorded (see Table 4). The compiled isotope data for each lake were used to calculate a mean isotope value for the gull's fish prey specific to each lake. These mean $\delta^{15}N$ values were used in the above equation to estimate gull trophic position.

For comparative purposes, $\delta^{15}N$ and $\delta^{13}C$ values were also measured in foods that herring gulls could obtain from anthropogenic sources, i.e., garbage. Samples of beef, chicken, and pork were purchased on three separate occasions in 1997 from different commercial sources in Ontario and Quebec. Each of the samples was processed and analysed in an identical manner as that described above. Mean $\delta^{15}N$ and $\delta^{13}C$ values were calculated for each type of meat ($n = 3$ for each type). $\delta^{15}N$ and $\delta^{13}C$ values were also measured in whole-body homogenates of three species of terrestrial birds. These individuals were collected in 1981–1982 from inland locations in southern Ontario. The species analysed had different diets (Ehrlich et al. 1988). Killdeers (*Charadrius vociferus*) ($n = 2$) are carnivores feeding primarily on insects, red-winged blackbirds (*Agelaius phoeniceus*) ($n = 3$) are omnivores with a diet consisting of insects and seeds, and rock doves (*Columba livia*) ($n = 3$) are herbivores feeding primarily on seeds. Although red-winged blackbirds may obtain a portion of their diet from aquatic sources, the samples originated from inland locations where terrestrial nitrogen and carbon inputs would be expected to be important. Regardless of whether or not herring gulls feed on these particular species, their isotopic signatures should be reflective of those found in terrestrial species in general. By analysing these species, we obtained a better idea of what isotopic signatures would be expected in potential terrestrial prey of herring gulls.

Statistical analysis

$\delta^{15}N$ and $\delta^{13}C$ values as well as our derived gull trophic position data were normally distributed (Shapiro–Wilk *W*) and colony variances were homogenous (Levene test). Intercolony differences in mean isotope values (1982–1995) were identified using ANOVA followed by Tukey's HSD test for unequal sample sizes (StatSoft 1995). Pearson correlations were used to examine temporal trends in $\delta^{15}N$ and $\delta^{13}C$ values and to investigate the relationship between $\delta^{15}N$ and $\delta^{13}C$ values within colonies. Spearman correlations were used to examine relationships between isotope data and measures of fish availability because relationships of this type are often nonlinear (Cairns 1987). The alpha level for all tests was 0.05.

Results

Pellet analysis

The composition of herring gull pellets collected from nests on Lake Superior was markedly different from those collected on Lakes Erie and Ontario (Table 1). On Lakes Erie and Ontario, pellets consisted primarily of fish. However, on Lake Superior, pellets contained a greater proportion of terrestrial food, namely garbage. The proportion of fish in the Lake Superior pellets was significantly lower than in pellets from Lakes Erie and Ontario (*G* test with a Williams correction, $p < 0.001$). While the proportion of fish in the diet of gulls from Lake Erie was greater than that observed on Lake Ontario, this difference was not statistically significant.

Table 2. $\delta^{15}N$ values (‰) in herring gull eggs collected from 1974 to 1995 from 13 Great Lakes colonies.

	Superior		Michigan		Huron			Detroit	Erie		Niagara	Ontario	
Year	1	2	3	4	5	6	7	8	9	10	11	12	13
1974	10.94												
1976	10.63												
1977													16.1
1978	10.17	10.54											14.85
1979		10.16											14.72
1980	10.32	10.91											14.43
1981	9.97	9.93											15.94
1982	9.56	9.77	12.03		12.63	10.17		12.63	17.68	16.63	16.9	15.31	14.75
1983		9.79	12.16	12.92	12.09	10.77	14.57	14.83	17.75	17.36	17.38	16.72	13.99
1984	10.5	10.79	13.75		13.6	10.54	15.73	14.84	18.63		17.55	14.85	14.99
1985	11.07	10.58	13.46	12.81	12.16	13.42	15.52	13.45	16.8	18.24	18.6	14.56	14.9
1986	10.87	10.72		12.11	12.44	11.64	15.32	13.46	18.95	16.25	18	14.21	13.81
1987	10.87	10.6	13.3	12.71	12.87	9.7	15.89	15.81	18.98	17.92	17.52	14.19	14.06
1988	11.15	10.88	14.18	13.49	12.64	11.99	15.64	13.74	18.23	17.46	18.7	14.13	15.32
1989	10.73	11.43	13.4	13.8	12.45	12.33	16.12	12.91	17.87	18.37	18.56	11.2	14.67
1990	10.01	10.42	13.34	11.9	12.61	7.37	16.31	12.82	18.43	14.94		14.16	13.72
1991	9.86	10.55	12.9	12.7	11.95	11.37	14.42	11.15	16.96	15.48	17.82	12.23	13.84
1992	10.42	11.65	9.91		12.23	11.93	16.13	14.16	17.14	18.3	18.3	14.4	14.04
1993	9.35	9.81	13.49	13.19	11.58	10.77	15.06	12.16	16.72	14.55	16.85	13.76	12.86
1994	10.23	10.5	13.53	10.83	11.64	10.22	14.05	13.18	16.41		16.04	14	13.12
1995	9.16	8.99	10.74	11.37	12.21	9.39	13.7	11.25	15.88	13.18	15.56	12.7	14.21
Mean	10.29	10.46	12.78	12.53	12.36	10.83	15.27	13.31	17.60	16.56	17.52	14.03	14.16
	f	*f*	*cde*	*de*	*e*	*f*	*b*	*cde*	*a*	*ab*	*a*	*bcd*	*bc*

Note: Colony arithmetic means were calculated using data from 1982 to 1995. Intercolony differences in mean $\delta^{15}N$ values are indicated by the letters beneath the means. Means with the same letter are not significantly different (Tukey's HSD test for unequal sample sizes).

Geographic differences in egg isotopic signatures

Mean $\delta^{15}N$ values for gull eggs differed among colonies (ANOVA, $F_{12,160} = 71.4$, $p < 0.001$). Annual $\delta^{15}N$ values from 1982 to 1995 are shown for each colony in Table 2. Lower $\delta^{15}N$ values were found in gull eggs from Lakes Superior, Huron (except Channel/Shelter Island), and Michigan (Tukey's HSD test for unequal sample sizes). Higher $\delta^{15}N$ values were measured in eggs from Lake Erie, the Niagara River, Channel/Shelter Island, Lake Ontario, and the Detroit River.

Mean $\delta^{13}C$ values also differed among colonies (ANOVA, $F_{12,160} = 11.5$, $p < 0.001$) (Table 3). In general, $\delta^{13}C$ was enriched in eggs from the lower Great Lakes (Tukey's HSD test for unequal sample sizes). However, egg $\delta^{13}C$ values showed more similarity among colonies than did egg $\delta^{15}N$ values.

Adjusting for interlake differences in baseline $\delta^{15}N$ signatures

Fish data used to calculate gull trophic positions are summarized in Table 4. Estimates of gull trophic position are summarized in Table 5. After adjusting for lake-specific differences in baseline $\delta^{15}N$ values, geographic differences in gull trophic level were less than those observed for the unadjusted $\delta^{15}N$ data. However, significant intercolony differences remained (ANOVA, $F_{12,160} = 25.8$, $p < 0.001$). For example, the trophic position occupied by gulls on Lake Erie and the Niagara River was higher than that of gulls on Lake Superior (Tukey's HSD test for unequal sample sizes).

Temporal changes in egg isotope signatures

Only isotope data from 1982 to 1995 were included in the examination of temporal trends. Earlier data were excluded because they were not available from most colonies. Statistically significant temporal declines in $\delta^{15}N$ values were observed in eggs from Double Island ($r^2 = -0.31$, $p = 0.04$), Middle Island ($r^2 = -0.37$, $p = 0.02$), Toronto Harbour ($r^2 = -0.36$, $p = 0.02$), and Snake Island ($r^2 = -0.32$, $p = 0.04$). Marginally significant declines were observed at Fighting Island ($r^2 = -0.28$, $p = 0.05$) and Port Colborne ($r^2 = -0.30$, $p = 0.06$).

The only area that exhibited a consistent change in egg $\delta^{13}C$ values was Lake Erie. In that region, egg $\delta^{13}C$ values increased through time at the Port Colborne ($r^2 = 0.56$, $p < 0.01$) and Niagara River ($r^2 = 0.40$, $p = 0.02$) colonies. A marginally significant increase in egg $\delta^{13}C$ values was observed in eggs from Fighting Island in the Detroit River ($r^2 = 0.27$, $p < 0.06$). For colonies on the other lakes, egg $\delta^{13}C$ values showed a significant increase at Agawa Rocks on Lake Superior ($r^2 = 0.29$, $p < 0.05$), Big Sister Island on Lake Michigan ($r^2 = 0.52$, $p = 0.01$), and Channel/Shelter Island on Lake Huron ($r^2 = 0.56$, $p < 0.01$). Other colonies on these lakes did not exhibit a significant temporal trend. There were no significant temporal trends in egg $\delta^{13}C$ values from Lake Ontario colonies. Colonies on Lake Erie and the Detroit River were the only locations at which a consistent change in both egg $\delta^{15}N$ and egg $\delta^{13}C$ values occurred over time.

To examine the possible reasons for the changes in isotopic signatures of eastern Lake Erie herring gull eggs, $\delta^{15}N$ and $\delta^{13}C$ values were measured in the most important prey fish species in that lake and in terrestrial prey and garbage (Fig. 2). $\delta^{15}N$ values for fish were significantly greater than those for any of the three types of garbage (beef, chicken,

Table 3. $\delta^{13}C$ values (‰) in herring gull eggs collected from 1974 to 1995 from 13 Great Lakes colonies.

	Superior		Michigan		Huron			Detroit	Erie		Niagara	Ontario	
Year	1	2	3	4	5	6	7	8	9	10	11	12	13
1974	–23.35												
1976	–23.08												
1977													–22.14
1978	–23.16	–22.16											–23.60
1979		–22.46											–22.06
1980	–23.07	–22.43											–22.25
1981	–21.99	–22.40											–21.99
1982	–22.57	–24.83	–25.30		–24.22	–23.48		–22.35	–23.69	–23.53	–22.97	–22.79	–24.20
1983		–24.99	–26.68	–26.09	–24.70	–24.08	–24.69	–23.38	–21.78	–23.09	–23.48	–20.85	–23.22
1984	–24.87	–24.26	–25.31		–23.94	–24.12	–24.12	–21.80	–22.51		–23.32	–20.85	–22.75
1985	–25.54	–24.33	–25.20	–26.25	–23.40	–25.15	–25.47	–21.59	–23.18	–24.27	–23.67	–20.92	–21.43
1986	–24.75	–23.43		–23.58	–22.29	–23.96	–23.63	–20.27	–23.06	–22.42	–23.15	–21.44	–23.10
1987	–24.86	–22.61	–24.30	–25.28	–23.24	–22.87	–24.05	–23.84	–24.17	–22.49	–22.47	–21.05	–22.75
1988	–23.30	–24.91	–24.71	–25.68	–23.65	–25.88	–25.08	–22.45	–23.42	–23.95	–24.14	–21.53	–24.58
1989	–24.55	–24.61	–26.40	–26.05	–24.18	–25.80	–24.76	–19.61	–23.93	–22.60	–23.56	–22.93	–23.37
1990	–24.18	–22.55	–26.69	–23.59	–24.09	–23.53	–24.11	–20.22	–21.96	–20.22		–22.37	–23.20
1991	–24.66	–23.48	–23.70	–24.23	–24.53	–25.52	–23.77	–20.76	–22.21	–20.23	–21.44	–21.57	–22.98
1992	–25.78	–25.84	–22.94		–24.51	–25.06	–24.05	–23.48	–21.59	–22.11	–22.03	–21.87	–24.44
1993	–21.57	–20.93	–23.97	–23.23	–23.02	–23.80	–22.88	–20.33	–22.51	–21.14	–23.46	–23.46	–22.87
1994	–24.43	–23.28	–26.33	–23.61	–24.46	–24.45	–22.17	–20.65	–22.25		–21.76	–22.48	–24.21
1995	–22.47	–21.28	–22.04	–23.55	–22.86	–22.18	–21.81	–18.92	–22.13	–20.41	–20.63	–20.08	–21.44
Mean	–24.12	–23.67	–24.89	–24.65	–23.79	–24.28	–23.89	–21.40	–22.74	–22.21	–22.78	–21.73	–23.18
	defg	*cdefg*	*g*	*fg*	*cdefg*	*efg*	*defg*	*a*	*abcd*	*abc*	*abcde*	*ab*	*bcdef*

Note: Colony arithmetic means were calculated using data from 1982 to 1995. Intercolony differences in mean $\delta^{13}C$ values are indicated by the letters beneath the means. Means with the same letter are not significantly different (Tukey's HSD test for unequal sample sizes).

Table 4. Prey fish species thought to be most important in the diet of herring gulls from each of the Great Lakes.

Lake	Species	Total length (mm)	Mass (g)	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	Source
Superior	Rainbow smelt*	114.4±3.4	6.3±0.4	8.32±1.67	–26.59±2.84	This study; Keough et al. 1996
	Lake herring			7.80	–26.20	Keough et al. 1996
	Mean			8.06±0.37	–26.40±0.28	
Michigan†	Alewife	145.2±4.4	21.2±3.1	9.17±0.20	–22.92±0.81	This study
Huron	Rainbow smelt	114.7±3.3	7.2±0.7	9.28±0.69	–24.72±0.24	This study
Erie	Freshwater drum	91.3±6.1	7.3±0.2	13.23±0.16	–21.62±0.13	This study
	Emerald shiner	79.3±2.7	3.3±0.2	12.33±0.48	–22.42±0.18	This study
	Gizzard shad	77.1±4.0	4.2±0.1	9.78±0.08	–21.39±0.11	This study
	Rainbow smelt	115.0±2.5	7.4±0.7	14.75±0.32	–22.03±0.34	This study
	White perch	74.2±10.3	4.0±0.7	12.97±0.18	–21.30±0.18	This study
	Yellow perch	149.1±22.2	39.6±15.6	13.62±0.48	–22.43±0.20	This study
	Mean			12.78±1.67	–21.86±0.50	
Ontario†	Alewife	140.9±7.3	19.3±5.9	13.02±0.13	–23.39±2.07	This study; Kiriluk et al. 1995

Note: Mean (±1 SD) $\delta^{15}N$ and $\delta^{13}C$ values are shown for each fish species. These fish values were used to estimate herring gull trophic position and allowed us to compensate for interlake differences in baseline isotope signatures. Where two or more fish species were deemed to be important constituents of the gull diet for a particular lake, a mean value was used to adjust herring gull egg $\delta^{15}N$ values. Mean (±1 SD) total length and mass of the fish analysed are also shown.

*Smelt isotopic values are means of values for smelt collected from the open lake and wetlands.

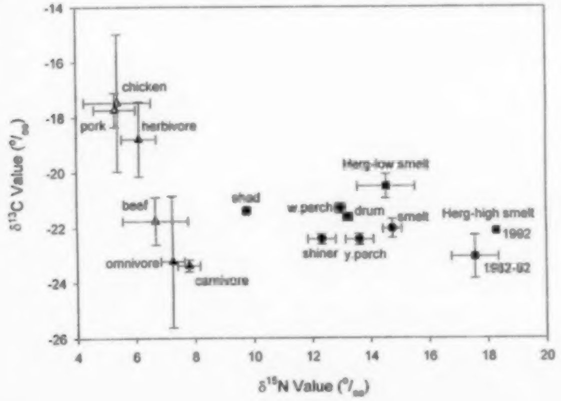
†For comparative purposes, smelt isotopic values from Lake Michigan are $\delta^{15}N = 10.42 \pm 0.26$ and $\delta^{13}C = -24.71 \pm 0.45$ and from Lake Ontario are $\delta^{15}N = 15.49 \pm 0.20$ and $\delta^{13}C = -24.38 \pm 0.41$. These values were not used in the calculation of gull trophic position because alewife was deemed to be the primary fish prey in the diet of gulls from these lakes.

Table 5. Trophic position of herring gull eggs collected from 1974 to 1995 from 13 Great Lakes colonies.

	Superior		Michigan		Huron			Detroit	Erie		Niagara	Ontario	
Year	1	2	3	4	5	6	7	8	9	10	11	12	13
1974	3.85												
1976	3.76												
1977													3.91
1978	3.62	3.73											3.54
1979		3.62											3.50
1980	3.66	3.84											3.41
1981	3.56	3.55											3.86
1982	3.44	3.50	3.84		3.99	3.26		3.47	4.44	4.13	4.18	3.67	3.51
1983		3.51	3.88	4.10	3.83	3.44	4.56	4.12	4.46	4.35	4.32	4.09	3.29
1984	3.72	3.80	4.35		4.27	3.37	4.90	4.12	4.72		4.37	3.54	3.58
1985	3.89	3.74	4.26	4.07	3.85	4.22	4.84	3.71	4.18	4.61	4.68	3.45	3.55
1986	3.83	3.78		3.86	3.93	3.69	4.78	3.71	4.81	4.02	4.50	3.35	3.23
1987	3.83	3.75	4.21	4.04	4.06	3.12	4.94	4.41	4.82	4.51	4.36	3.34	3.31
1988	3.91	3.83	4.47	4.27	3.99	3.80	4.87	3.80	4.60	4.38	4.71	3.33	3.68
1989	3.79	3.99	4.24	4.36	3.93	3.90	5.01	3.55	4.50	4.64	4.66	2.46	3.49
1990	3.57	3.69	4.23	3.80	3.98	2.44	5.07	3.53	4.66	3.64		3.34	3.21
1991	3.53	3.73	4.10	4.04	3.79	3.61	4.51	3.04	4.23	3.79	4.45	2.77	3.24
1992	3.69	4.06	3.22		3.87	3.78	5.01	3.92	4.28	4.62	4.59	3.41	3.30
1993	3.38	3.51	4.27	4.18	3.68	3.44	4.70	3.33	4.16	3.52	4.16	3.22	2.95
1994	3.64	3.72	4.28	3.49	3.69	3.28	4.40	3.63	4.07		3.92	3.29	3.03
1995	3.32	3.27	3.46	3.65	3.86	3.03	4.30	3.06	3.91	3.12	3.78	2.91	3.35
Mean	3.66	3.71	4.06	3.99	3.91	3.46	4.76	3.67	4.42	4.11	4.36	3.30	3.34
	def	*cde*	*bcd*	*bcd*	*cd*	*ef*	*a*	*def*	*ab*	*bc*	*ab*	*f*	*ef*

Note: Colony arithmetic means were calculated using data from 1982 to 1995. Intercolony differences in mean trophic position are indicated by the letters beneath the means. Means with the same letter are not significantly different (Tukey's HSD test for unequal sample sizes).

Fig. 2. Mean $\delta^{15}N$ (± 1 SD) and mean $\delta^{13}C$ (± 1 SD) values in Port Colborne herring gull eggs (Herg) from years of high (1992 only or 1982–1992) and low (1990, 1991, 1993, and 1995) rainbow smelt abundance. Mean $\delta^{15}N$ (± 1 SD) and mean $\delta^{13}C$ (± 1 SD) values are also shown for a variety of potential fish prey species (●) collected from Lake Erie and for foods from terrestrial (birds, ▲) and anthropogenic sources (garbage, △). Values in these last two categories are not specific to Lake Erie.



and pork) or for any of the three avian species (ANOVA, $F_{6,30} = 36.1$, $p < 0.0001$; Tukey's HSD test for unequal sample sizes). $\delta^{13}C$ values also exhibited differences among the potential food items (Kruskal–Wallis test, $H = 23.1$, $p < 0.001$). $\delta^{13}C$ values were significantly greater in chicken and pork than in fish (Mann–Whitney *U* test, $U = 0.0$, $p = 0.006$). The herbivorous birds also had greater $\delta^{13}C$ values than the fish (Mann–Whitney *U* test, $U = 0.0$, $p = 0.006$). $\delta^{13}C$ values in beef and in the two other bird species were not significantly different from the values measured in fish.

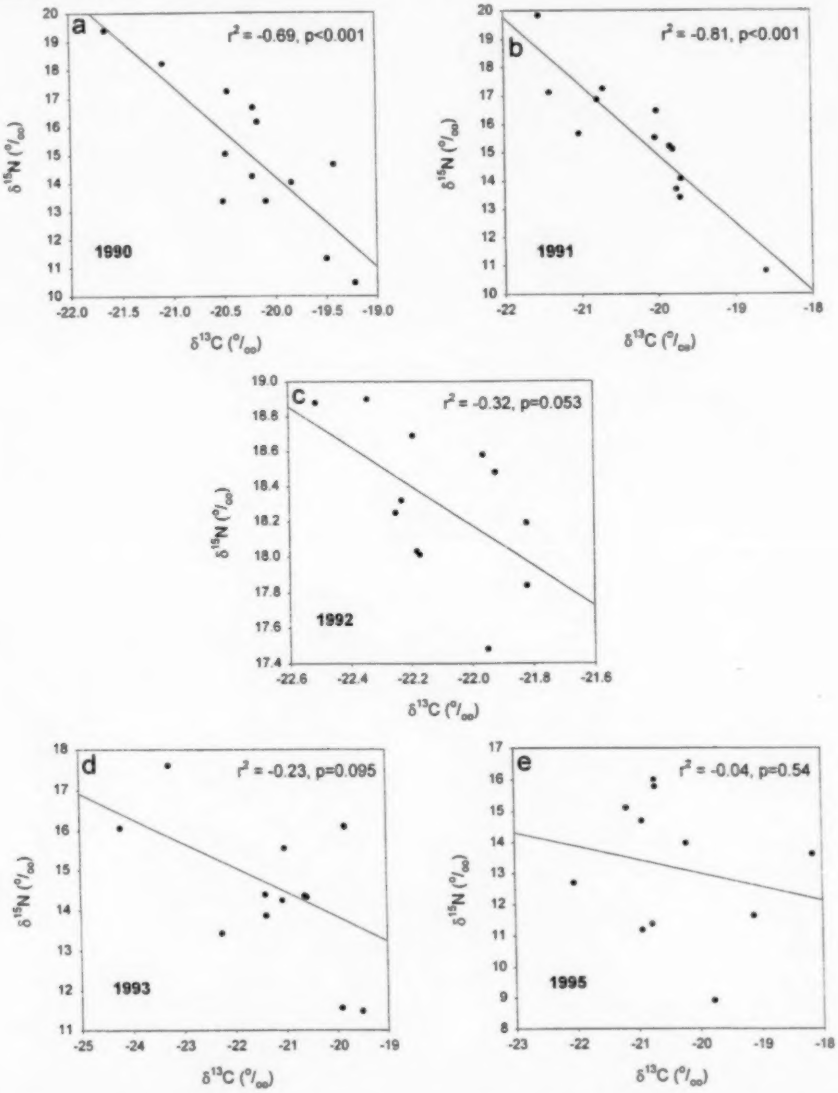
Relationship between gull trophic position and terrestrial feeding

Significant negative relationships were found between annual mean egg $\delta^{15}N$ and egg $\delta^{13}C$ values at nine of 13 colonies (r^2 ranged from –0.31 to –0.59, $p < 0.05$). Significant negative relationships were observed at all colonies except Double Island, Middle Island, and the two Lake Ontario colonies. To further examine this negative relationship between egg $\delta^{15}N$ and $\delta^{13}C$, we examined individual egg analyses for the Port Colborne colony from 1990 to 1993 and 1995 (Fig. 3). Results from Middle Island are not presented because variations in egg $\delta^{13}C$ values were small (Table 3). At Port Colborne, in contrast, there was considerable variability in both $\delta^{13}C$ and $\delta^{15}N$ within years. For example, in 1990 and 1991, egg $\delta^{15}N$ values ranged from about 10 to 20‰ and $\delta^{13}C$ ranged from –22 to –19‰. For two of the five years (1990 and 1991), there was a significant negative relationship between $\delta^{15}N$ and $\delta^{13}C$ values (Figs. 3*a* and 3*b*). In 1992 and 1993, marginally significant relationships were observed (Figs. 3*c* and 3*d*).

Discussion

Our study revealed considerable isotopic variation in her-

Fig. 3. Relationship between egg $\delta^{15}N$ and $\delta^{13}C$ for individual herring gull eggs collected from the Port Colborne colony on Lake Erie in (*a–d*) 1990–1993 and (*e*) 1995.



ring gull eggs among colonies and through time for some locations. Several factors may have contributed to these results; they include isotopic differences among lakes as well as real dietary differences in gulls within and between lakes.

Trophic enrichment in ^{15}N has been shown to occur in the Lake Ontario (Kiriluk et al. 1995) and Lake Superior food webs (Keough et al. 1996). In this study, we found that the $\delta^{15}N$ signatures of the prey fish species most likely consumed by gulls ranged from 8 to 13‰ (see Table 4). Gulls feeding exclusively on these fish species are expected to lay eggs with $\delta^{15}N$ values ranging from 11.4 to 16.4‰ (Hobson 1995). The scavenging of fish offal derived from fisheries of piscivorus fish (e.g., lake trout (*Salvelinus namaycush*), walleye (*Stizostedion vitreum*) would provide foods for gulls that could in turn result in eggs with even greater $\delta^{15}N$ values (Kiriluk et al. 1995; Keough et al. 1996).

Predicting $\delta^{15}N$ values corresponding to the broad range of terrestrial foods available to gulls is problematic, since no detailed isotopic inventory of such foods has been made. We also recognize that predicting terrestrial $\delta^{15}N$ values is difficult because of complicating factors such as ammonification and the use of fertilizers in agriculture. However, many foods, such as terrestrial vegetation, herbivorous invertebrates, small mammals, and songbirds, likely have lower $\delta^{15}N$ values than those of higher trophic level planktivorous or piscivorus fish in the Great Lakes. Support for this argument was provided by our analysis of avian species utilizing terrestrial food sources. We found that the $\delta^{15}N$ values in herbivorous, omnivorous, and insectivorous birds were significantly lower than the values observed in Great Lakes fish. Additional data are available from archaeological investigations of faunal remains from the Kelly Campbell site near Lake Huron. Katzenberg (1988) measured stable isotope ratios in bone collagen from six species of upland terrestrial mammals and birds. δ^{15}N in the faunal remains ranged from 1.1 to 11.9‰ (mean 6.2 ± 2.5‰, *n* = 17). Applying an expected diet to collagen isotopic fractionation factor of 3‰ for δ^{15}N values indicates that terrestrial systems exploited by gulls may be less enriched in ^{15}N (i.e., a mean δ^{15}N value of 6.2 – 3 = 3.2‰) than fish from all of the Great Lakes. The stable isotope values of garbage are expected to be diverse and are difficult to predict, but most of the meat products found in garbage should originate from herbivorous domesticated animals, e.g., poultry, cattle, swine, and sheep. Although commercial feeds may include animal protein, the contribution to total protein in the diet is low. For example, animal protein in the diet of domestic swine never exceeds 5–7% (Knabe 1996). In this study, the δ^{15}N values measured in food/protein that could be available to gulls through garbage were lower than the δ^{15}N signatures of fish from Lake Erie (Fig. 2) or the other Great Lakes (Table 4). Therefore, with an increasing proportion of terrestrial items in the gull diet, egg δ^{15}N values would be expected to decrease.

Using data from this study and others, we found that the primary prey fish species consumed by herring gulls on the Great Lakes had δ^{13}C signatures ranging from about –22 to –26‰ (see Table 4). We have less information regarding the stable carbon isotopic composition of terrestrial diets of gulls. In this study, differences in δ^{13}C between terrestrial birds and fish from Lake Erie were not consistent. In two of the terrestrial bird species (killdeer and red-winged blackbird), δ^{13}C values were not significantly different from the δ^{13}C values measured in Lake Erie fish. Conversely, δ^{13}C values in the herbivorous bird species (rock dove) were greater than the fish δ^{13}C values. These results must be considered in light of the fact that δ^{13}C values in Lake Erie fish were more enriched in ^{13}C than in the other lakes (Table 4). Therefore, in the other lakes, particularly Lakes Superior and Huron, the relative enrichment of ^{13}C in terrestrial birds compared with fish would be more apparent. For example, the δ^{13}C values in Lake Superior rainbow smelt and lake herring are well below those measured in all three bird species (see Fig. 2 and Table 4). Katzenberg's (1988) measurements of stable carbon isotope ratios in bone collagen from upland terrestrial species resulted in a mean δ^{13}C value of –21.7 ± 1.6‰ (*n* = 17). However, stable carbon isotopic fractionation between diet and bone collagen is on the order of +5‰ (van der Merwe 1982), whereas the corresponding value between diet and muscle tissue is on the order of 1‰ (Peterson and Fry 1987). This suggests that, under conditions of constant diet, collagen and meat δ^{13}C values in the same individual will likely differ by about 4‰. Thus, the δ^{13}C value of the dietary meat component of the upland terrestrial mammals and birds from this archaeological site would be of the order of –25.7‰, a value close to that for aquatic prey. France (1995*a*, 1996) similarly pointed out an overlap in δ^{13}C values between upland and aquatic food webs based upon attached algae. However, other studies have indicated that lentic phytoplankton are more depleted in ^{13}C than attached algae or allochthonous carbon inputs (Rau 1980; France 1995*a*). For this reason, we anticipate that terrestrial foods could be enriched in ^{13}C compared with food originating from phytoplankton-based aquatic food webs. This would certainly be the case if any C-4 derived foods were available through human foods at garbage dumps (Nakamura et al. 1982; also see Hobson 1987). In this study, δ^{13}C values in protein (beef, chicken, and pork) from anthropogenic sources were enriched in ^{13}C compared with potential fish prey from the Great Lakes (with the exception of δ^{13}C values in beef and Lake Erie fish) (see Fig. 2 and Table 4).

The significant negative relationships observed at most colonies between annual measurements of egg δ^{15}N and δ^{13}C values may have reflected annual differences in the proportion of the herring gull diet consisting of aquatic versus terrestrial prey. This hypothesis was supported by the results of our analysis of individual herring gull eggs from Port Colborne for specific years. For four of the five years, there was a negative relationship between egg δ^{15}N and δ^{13}C values. Therefore, the relationship is consistent at different temporal scales. Intracolony variation in isotope values for individual years at Port Colborne indicates that there are differences in the foraging habits of individual birds. The degree to which this variation arises may be the result of changes in the availability of high-quality food, i.e., fish, from one year to the next.

While further isotopic measurements of food webs associated with herring gulls on the Great Lakes are clearly required, on the basis of the above results, the following working hypothesis can be presented: greater δ^{15}N and lower δ^{13}C values in gull eggs, when considered either spatially or temporally, correspond to increases in the proportion of fish in gull diets. Below, we present correlational evidence from fisheries data to support this hypothesis.

Geographic differences

Recent research in Lake Ontario has indicated that seasonal differences in the δ^{13}C signature of particulate organic matter may occur as a result of seasonal changes in the rate of primary productivity (Leggett et al. 1997). According to this theory, as primary production increases, the amount of dissolved CO_2 in the water decreases. This results in an influx of ^{13}C-enriched atmospheric CO_2 into the lake, causing the carbon isotope signature of primary producers to become more enriched in ^{13}C. This mechanism may also be operating on a geographic scale, given that there are large interlake differences in primary production. Baseline δ^{13}C signatures are expected to be somewhat greater in the lower Great Lakes as a result of this mechanism, and this could have led to the greater δ^{13}C values observed in eggs from the lower Great Lakes.

Another factor potentially contributing to the greater than expected similarity of egg δ^{13}C values among lakes is differences in the composition of the fish communities among the lakes. Different prey fish have been found to have different δ^{13}C signatures. For example, δ^{13}C values in alewife from Lake Ontario are slightly more enriched in ^{13}C than are rainbow smelt from the same lake (Kiriluk et al. 1995). Therefore, in Lake Superior, where smelt are an important prey fish, the δ^{13}C signature of the gull's fish diet is expected to

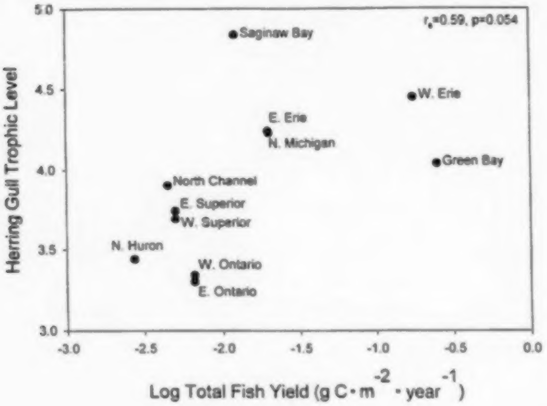


Fig. 4. Relationship between median trophic position of herring gull eggs collected from 1982 to 1995 and total fish yield for various Great Lakes locations. Fish yield data are from Rowan and Rasmussen (1992).

be more negative than in Lake Ontario where the major prey fish species is alewife.

Differences in gull trophic position among colonies also reflected differences in the diets of herring gulls from those colonies. Previous studies using pellets, boli, and faeces have indicated that herring gulls from Lakes Erie and Ontario have a larger proportion of fish in their diets than birds from Lakes Superior and Huron (Fox et al. 1990; Ewins et al. 1994). Pellets analysed in this study confirm these earlier results. There was a greater incidence of fish in pellets collected from Lakes Erie and Ontario than from Lake Superior.

Intercolony differences in the proportion of fish in the herring gull diet may be explained by the amount of primary production occurring in the vicinity of breeding colonies. In locations where primary production is greater, fish yield/production may also be greater (LeCren and Lowe-McConnell 1980). As fish biomass and numbers increase, the likelihood of encounters between gulls and fish will also increase. Fish are a preferred prey type from both a caloric and nutritional perspective (Pierotti and Annett 1987; Shutt and Hebert 1996). However, gulls may optimize their foraging strategies to maximize nutritional intake and minimize the energetic costs of foraging (Ewins et al. 1994). As fish become more abundant, the foraging costs associated with pursuing fish are also reduced, making them more profitable from an energetic perspective.

Rowan and Rasmussen (1992) summarized data regarding primary production and fish yield for a variety of locations within the Great Lakes. Fish yield was used as a surrogate for fish production because of the lack of available production data for many areas. Although yield data may be biased because of differences in the amount of fishing effort expended among areas, the fact that log-transformed primary production and fish yield were positively correlated (r^2 = 0.59, p = 0.002) suggests that fish yield is a reasonable indicator of geographic differences in fish production and, hence, fish availability to gulls. We examined the relationship between fish availability and the gull diet by plotting Rowan and Rasmussen's (1992) summaries of log total fish yield against median trophic level of gull eggs taken from colonies located in similar areas. A marginally significant correlation was observed between total fish yield and gull trophic position (r_s = 0.59, p = 0.053) (Fig. 4). The correlation between this δ^{15}N-derived endpoint and total fish yield indicates that gulls were acting as integrators of aquatic ecosystem processes. Their utilization of aquatic food resources was not random but, in part, reflected differences in fish abundance/availability brought about by differences in biological productivity at lower trophic levels.

Temporal changes

In addition to potentially complicating the interpretation of geographic trends, changes in baseline isotope signatures through time, as a result of oligotrophication, could affect the interpretation of temporal trends in δ^{15}N and δ^{13}C values. In this study, we found that intrayear isotope values in eggs from one colony on Lake Erie showed considerable variation (Fig. 3). Obviously, differences among eggs from the same colony during the same year were not the result of differences in baseline isotopic signatures. Instead, they likely resulted from differences in feeding behaviour among individual birds. In addition, there are temporal fluctuations in isotope values that do not follow a consistent temporal trend. For example, in 1992, δ^{15}N values in Port Colborne eggs were much greater than values in eggs from immediately preceding or succeeding years (Table 2). This large increase in δ^{15}N values in Port Colborne eggs in 1992 may have reflected a change in the availability of the major prey fish species in the eastern basin of Lake Erie (see below). Given these results, we believe that the isotopic temporal trends described in this paper primarily reflect changes in the diet of the herring gull. Because large-scale geographic differences in fish yield/production and egg isotope values were correlated, it is logical to examine temporal changes in gull egg isotope signatures in terms of temporal trends in the abundance (biomass) of the primary prey fish species that the gulls were consuming.

Changes in gull diet from the lower Great Lakes were more dramatic than those observed throughout the rest of the Great Lakes basin. Of all of the colonies, only those on or near Lake Erie exhibited a consistent temporal change in both egg δ^{15}N and egg δ^{13}C values. As mentioned previously, profound ecological changes have occurred in the Lake Erie ecosystem in recent years (Leach 1993; Nicholls and Hopkins 1993; Howell et al. 1996). For that reason, we focus on explaining the trends in that lake.

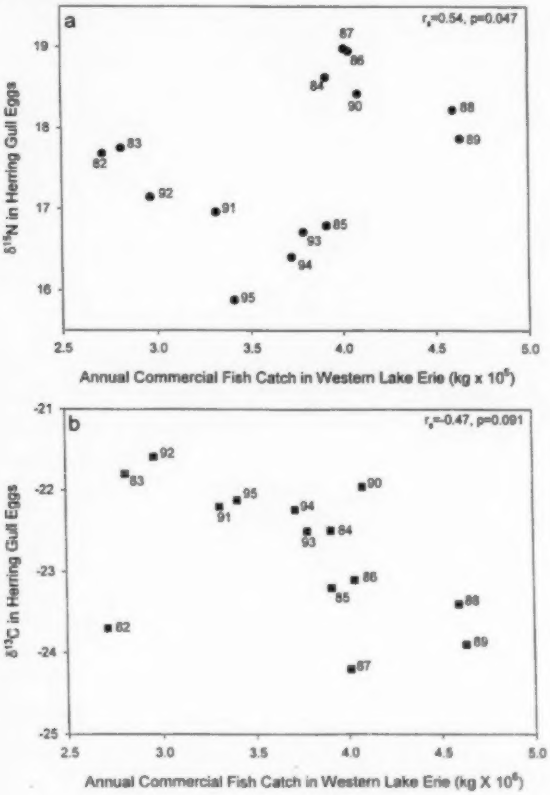
Lake Erie

On Lake Erie, increases in egg δ^{13}C occurred at Port Colborne, in the Niagara River, and in the Detroit River. Declines in egg δ^{15}N (and therefore, gull trophic position) were observed in the Detroit River as well as in both the western and eastern basins of the lake.

Western basin

Indices of abundance for the major prey fish species in the western basin of Lake Erie (Ontario Ministry of Natural Resources (OMNR) 1995) did not correlate with gull egg isotope values (all species, p > 0.10). However, the degree to

Fig. 5. Relationship between annual isotope values (‰) in herring gull eggs from Middle Island and the total commercial fish catch from the Canadian waters of western Lake Erie. Fish catch estimates are from OMNR management area OE-1 (OMNR 1982–1995). (*a*) δ^{15}N; (*b*) δ^{13}C; numbers beside symbols represent year.



which commercial fishing operations are active in waters surrounding the colony likely affected the availability of fish to herring gulls. Egg δ^{15}N and δ^{13}C values were correlated with annual estimates of the total commercial harvest from Ontario waters of western Lake Erie (management area OE-1 statistics summarized in OMNR 1982–1995). With increasing total fish harvest, egg δ^{15}N values increased and δ^{13}C values declined (Fig. 5). Gull trophic position, as inferred from eggs, in the western basin (Middle Island) was greater than that predicted (trophic position 4) if birds consumed prey fish (e.g., smelt) entirely (Table 5). This might have resulted from our assumption that the fish consumed by gulls occupied trophic level 3 when, in fact, their actual trophic position was somewhat greater than 3 (see Vander Zanden and Rasmussen 1996). Alternatively, the fact that gull trophic position was greater than 4 from many years suggests that at least part of their diet may have consisted of high trophic level piscivorus fish. These are species that herring gulls would rarely consume if they were only obtained via natural foraging. However, a valuable commercial fishery exists in western Lake Erie, with the primary commercial species being the piscivorus walleye (OMNR 1982–1995). Therefore, it appears that in areas where commercial fishing operations for high trophic level predators are active, and gull trophic position is greater than 4, fishery-related sources of fish (discards, processing waste, opportunistic predation of fish in nets) are likely important constituents of the gull diet. Results from our study indicate that this may only be the case in northern Lake Michigan, Saginaw Bay, and Lake Erie. Results for Saginaw Bay should be treated with caution, however, as the fish isotopic signatures used to adjust gull eggs from Lake Huron were those for fish collected in the main part of the lake. δ^{15}N signatures of fish from Saginaw Bay might be greater than those in fish from the main lake because of anthropogenic inputs into the highly urbanized/industrialized Saginaw Bay watershed (Cabana and Rasmussen 1996). If this was the case, then the trophic level of Saginaw Bay gulls was overestimated. Nonetheless, our analysis suggests that where commercial fishing operations are active, they may play an important role in determining the composition of the herring gull diet. The importance of fishing activities in affecting the availability of fish to gulls has been documented in other areas (Oro et al. 1996). It is likely that much of the fish protein obtained by gulls from commercial fishing operations is in the form of fish offal. Traditional methods of assessing avian diets using pellets or faeces would not have detected this contribution of high trophic level fish to the gull diet because no evidence would remain of these easily digested foods. This underlines the value of using both stable isotopes and conventional approaches in assessing the composition of avian diets.

Eastern basin

The greatest temporal declines in gull trophic position occurred in the eastern basin (Port Colborne). To interpret these trends, we examined how rainbow smelt abundance has changed through time in that region. Rainbow smelt are the primary prey fish species in the eastern basin (OMNR 1995); therefore, trends in smelt abundance should have an important effect on the proportion of fish in the gull diet. Estimates of smelt abundance in the eastern basin (Pennsylvania Fish Commission 1996) were correlated with egg δ^{15}N ($r_s = 0.70$, $p = 0.011$) and δ^{13}C ($r_s = -0.73$, $p = 0.007$) but not linearly (Fig. 6). The shape of the δ^{15}N versus smelt relationship was similar to other seabird endpoints proposed as indicators of marine food availability, e.g., adult survivorship, adult colony attendance, chick growth, breeding success (Cairns 1987). Smelt are the most important commercially harvested species in the eastern basin of Lake Erie. For that reason, we also examined the relationship between the isotopic signatures of gull eggs from Port Colborne and annual harvest statistics for the Ontario portion of eastern Lake Erie (management area OE-5 statistics summarized in OMNR 1982–1995) (Fig. 7). It is interesting to note that the significant relationships observed were very similar to those found using the abundance estimates from Pennsylvania (Fig. 6). In this situation, where the main commercial species is also the most locally abundant prey fish, it is impossible to determine what role increased fishing activities had on gull isotope signatures. Presumably, fishing effort and harvest increase with increasing smelt abundance; therefore, increases in the proportion of fish in the gull diet

Fig. 6. Relationship between annual isotope values (‰) in herring gull eggs from Port Colborne and estimates of rainbow smelt abundance from eastern Lake Erie. Smelt data are from trawling surveys conducted by the Pennsylvania Fish Commission (1996). (*a*) $\delta^{15}N$; (*b*) $\delta^{13}C$; numbers beside symbols represent year.

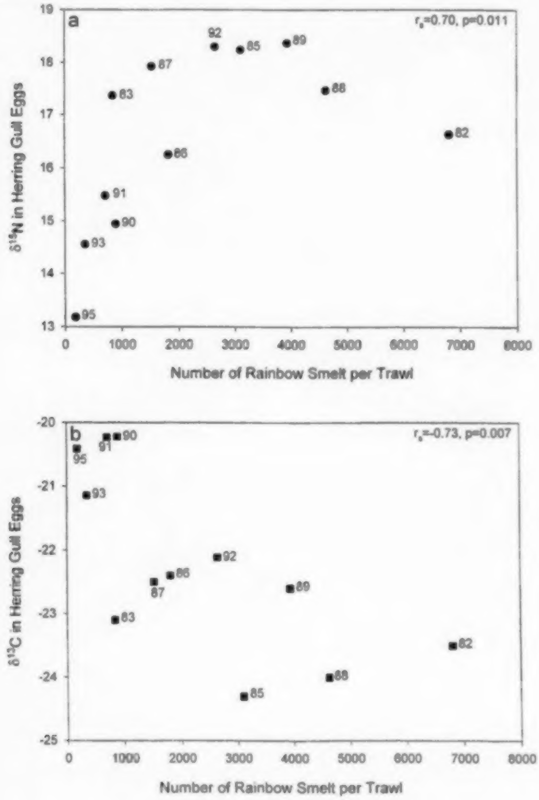
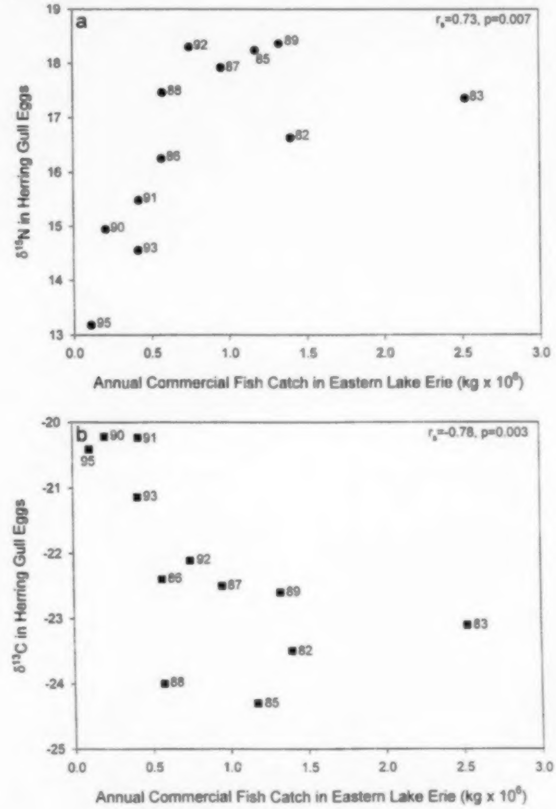


Fig. 7. Relationship between annual isotope values (‰) in herring gull eggs from Port Colborne and the total commercial fish catch from the Canadian waters of eastern Lake Erie. Fish catch estimates are from OMNR management area OE-5 (OMNR 1982–1995). (*a*) $\delta^{15}N$; (*b*) $\delta^{13}C$; numbers beside symbols represent year.



may have been affected by two factors: (*i*) increased prey fish abundance leading to a greater probability of gull–smelt predatory encounters and (*ii*) increased commercial fishing activity, which may have resulted in an increased availability of fishery "waste". Regardless, both of these mechanisms will be affected by factors that change smelt abundance.

Annual fluctuations in smelt abundance are evident through time, e.g., 1992, and have been attributed to density-dependent interactions within the smelt population (Henderson and Nepszy 1989). Long-term declines in smelt abundance may be related to reduced ecosystem productivity, salmonid stocking, and commercial fishing operations (Henderson and Nepszy 1989). Recent dramatic changes in the Lake Erie ecosystem suggest that dreissenid mussels may also be playing a role in the decline of rainbow smelt populations. The zebra mussel (*Dreissena polymorpha*) was first identified in Lake St. Clair in 1988. From there, it spread eastward into the western and eastern basins of Lake Erie, arriving in 1988 and 1989, respectively (Mackie 1991; Howell et al. 1996). The introduction of the zebra mussel was soon followed by a related species, the quagga mussel (*Dreissena bugensis*) (May and Marsden 1992). In recent years, water chlorophyll *a* concentrations and plankton abundance have declined, Secchi disk transparencies have increased, and benthic community structure has been altered (Leach 1993; Nicholls and Hopkins 1993; Howell et al. 1996). These changes coincided with the arrival of dreissenid mussels into the Lake Erie ecosystem. The role of dreissenid mussels in redirecting primary production from pelagic to benthic systems has been documented (Fahnenstiel et al. 1995). Declines in smelt abundance/catch in the eastern basin are coincident with increases in mussel densities.

As the number of dreissenid mussels increased, competition for food resources likely intensified, resulting in reduced food for planktivorous fish such as smelt. This may have been more pronounced in the eastern basin of the lake because it is less productive (Neilson et al. 1995). In the eastern basin, where the fish community is dominated by rainbow smelt, there may have been less resilience to the redirection of pelagic production to the benthos by dreissenid mussels than in the western basin, where the fish community is more diverse (OMNR 1995). A decrease in smelt abundance may have resulted in greater declines in gull trophic

Fig. 8. Difference in the (*a*) δ^{15}N and (*b*) δ^{13}C values of rainbow smelt and other potential herring gull food types. Birds indicate the difference that might be expected in isotope values between smelt and natural, terrestrial food sources. Garbage represents foods that gulls could obtain from terrestrial, anthropogenic sources. The broken line shows the difference in herring gull egg δ^{15}N and δ^{13}C values between years of high (1992) and low (1990, 1991, 1993, and 1995) smelt abundance. Error bars represent 1 SD.

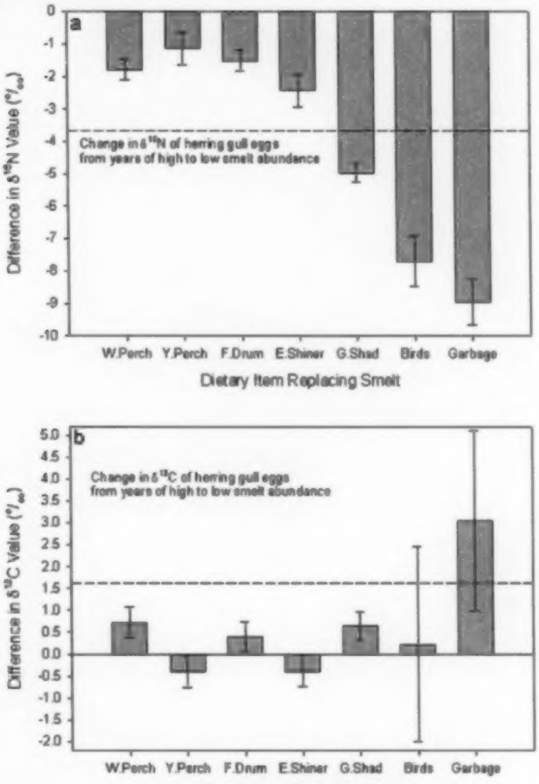
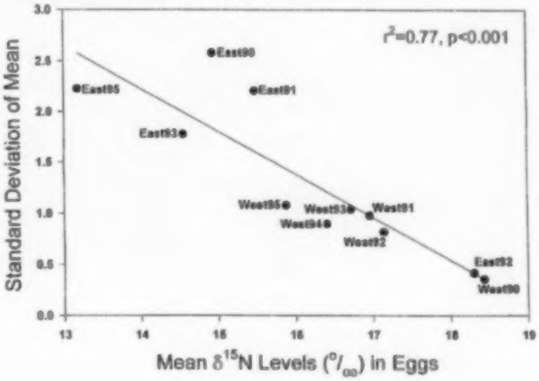


Fig. 9. Correlation between mean δ^{15}N values (1990–1995) in Lake Erie herring gull eggs and the degree of variability in the gull diet as indicated by the standard deviation of the mean. Colonies include Middle Island (West) and Port Colborne (East). Numbers represent year of collection.



position and increases in the proportion of the gull diet originating from terrestrial sources in gulls from the eastern basin. In addition, the commercial fishery in the eastern basin is much smaller than that in the western portion of the lake. This probably resulted in the birds from the eastern basin having fewer opportunities to capitalize on fish made available through these operations, and therefore, they relied to a greater extent on obtaining fish through more "natural" foraging methods.

Although most pronounced in Lake Erie, the impact of dreissenid mussels may have also contributed to the declines in δ^{15}N values for eggs at other colonies. For example, dreissenid mussels have had a profound effect on the Saginaw Bay ecosystem (for an overview, see Nalepa and Fahnenstiel 1995). Mussels were first found in large numbers in 1991. Since that time, δ^{15}N values in gull eggs have declined and δ^{13}C values have increased (Tables 2 and 3). Similar phenomena may be occurring in other parts of the Great Lakes, e.g., Lake Ontario (Stewart and Haynes 1994).

In eastern Lake Erie, declines in smelt availability probably affected the δ^{15}N and δ^{13}C values of herring gull eggs (Fig. 6). Consequently, it is logical to question whether the magnitude of these changes in egg isotope values could be explained by shifts in the gull diet from smelt to other prey fish species. To avoid the potential complications associated with temporal changes in nutrient loadings and lake productivity (see above), only the most recent years (1990–1995) were used in the examination of this question. However, similar results would have been obtained if all years of high smelt availability had been used (see mean δ^{15}N and δ^{13}C values in gull eggs from 1992 compared with means for all years of high smelt abundance, Fig. 2). Examination of the magnitude of the difference in gull egg δ^{15}N and δ^{13}C values between years of high (1992) and low (1990, 1991, 1993, and 1995) smelt abundance indicated that the differences in the isotopic signatures of smelt and other potential fish prey were insufficient to be responsible for the degree of change observed in the gull eggs (except for gizzard shad with regard to δ^{15}N) (Fig. 8). However, the change in egg isotope values could be explained by a mixed diet of fish, garbage, and perhaps other terrestrial foods (as indicated by the terrestrial bird isotope signatures). These results certainly suggest that gulls relied on terrestrial foods to a greater extent when smelt availability was low.

Examination of the mean egg δ^{15}N values calculated annually for both Lake Erie colonies (1990–1995) revealed that as mean egg δ^{15}N values increased, the standard deviation of the mean decreased (Fig. 9). This relationship provided evidence that when fish were available, gulls preferentially chose them as their primary food source. However, as fish became less available the gulls were forced to consume more terrestrial prey. A switch to increasing proportions of terrestrial foods would have resulted in a decline in mean egg δ^{15}N values and an increase in the standard deviation of the mean as foods with a wider variety of δ^{15}N signatures were consumed (see Fig. 2). This process may have been particularly important in the eastern basin where the degree of diet variability was much more pronounced. It is also interesting to note that the standard deviations at both colonies were greater in the most recent years, indicating that fish availability may be decreasing further. With continued declines in fish availability, further reductions in the proportion of fish in the herring gull diet would be expected. As suggested above, gulls may be able to switch to other food types when presented with this dilemma; however, it is unlikely that replacement foods are as nutritious (Shutt and Hebert 1996). This may be of particular importance in the eastern basin where the proportion of fish in the gull diet has already decreased substantially. In addition, obligate avian piscivores, such as terns, may be presented with greater difficulties as a result of prey fish scarcity.

Fish availability to Great Lakes herring gulls may be affected by spatial differences in fish abundance that reflect geographic differences in aquatic production. Commercial fishing operations may also lead to increased fish availability to some Great Lakes gulls. In addition, Hebert et al. (1997) found that changes in the proportion of fish in gull diets on Lake Ontario were partly regulated by winter severity and resultant alewife mortality. In severe winters, alewife mortality may increase, leading to a greater proportion of fish in the gull diet. Additional support for this hypothesis was provided by the $\delta^{15}N$ values in gull eggs from Snake Island (eastern Lake Ontario) in the spring of 1977. During the winter of 1976–1977, a massive die-off of alewife occurred, dramatically reducing their abundance (O'Gorman and Schneider 1986). It is probable that these dead/dying fish were more available to foraging gulls, resulting in the greatest $\delta^{15}N$ egg values ever observed at that colony (Table 2). Gull predation on dead/dying alewife affected by the severe 1983–1984 winter was observed in the offshore waters of Lake Ontario in February 1984 (R. O'Gorman, Oswego Biological Station, 17 Lake St., Oswego, N.Y., personal communication). As a result of this weather-mediated change in diet composition, levels of persistent contaminants increased in gull eggs. This probably occurred because fish, as a result of their higher trophic position, are more contaminated than other dietary items. Levels of persistent organochlorines increase from one trophic level to the next as a result of food chain biomagnification (Oliver and Niimi 1988). A pertinent result from this study is the identification of spatial and temporal differences in the trophic position of gulls from the Great Lakes. How these differences influence intercolony and interyear differences in contaminant exposure and accumulation is currently being assessed.

Conclusions

In this study, we have shown that the proportion of fish in the gull diet may be affected by two primary factors: the abundance of fish species, as influenced by production at lower trophic levels, and the enhanced availability of fish as a result of commercial fishing activities. In addition, fish availability may be affected by the susceptibility of fish species to external factors such as overwinter mortality. These last two factors may only be important in areas of the Great Lakes where commercial fishing activity is high (Lake Erie, northern Lake Michigan) or where the prey fish community is dominated by exotic species that have not evolved to withstand the harsh weather of the Great Lakes region (e.g., Lake Ontario, Lake Michigan).

Future trends in the isotopic signatures of aquatic bird tissues and their prey will provide additional information on the availability of fish to high trophic level predators in the Great Lakes. Fish-eating birds act as integrators of ecosystem processes, and changes in the composition of their diet resulting from alterations in pathways of energy flow may be indicative of stress in aquatic systems (see Ulanowicz 1996). Although the Herring Gull Monitoring Program was specifically designed to track contaminant trends in the Great Lakes, this study has demonstrated its utility in providing insights into Great Lakes food webs.

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Impact of wildfire and clear-cutting in the boreal forest on methyl mercury in zooplankton

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Abstract: We compared the effects of forest logging and wildfire on methyl mercury (MeHg) concentrations in bulk zooplankton collected in 38 oligotrophic lakes located in the boreal forest. Mean MeHg concentrations were slightly but significantly higher ($P < 0.01$) in zooplankton collected in lakes with recently logged watersheds (135 ng·g dry weight^{-1}) than in lakes with recently burned (97 ng·g dry weight^{-1}) or undisturbed watersheds (112 ng·g dry weight^{-1}). MeHg levels in zooplankton collected in burned and reference lakes were not significantly different. Multiple regression including all the lakes showed that four variables (dissolved organic carbon, pH, dissolved oxygen, and immature zooplankton biomass) explained 43% of the variability in MeHg levels in bulk zooplankton. Regressions calculated for each group of lakes explained less (reference lakes) or more of the variability in MeHg (logged and burned lakes) than the general model did. Dissolved organic carbon was the only variable common to all models, which indicates the importance of watershed processes in the transfer of humic matter bound Hg to lakes. However, variables related to methylation rate, such as pH, sulfate concentrations, and average dissolved oxygen in lake water, also explained part of the variation in MeHg concentrations in zooplankton.

Résumé : Nous avons comparé les effets de la coupe forestière et des feux de forêt sur les teneurs en méthyl mercure (MeHg) du zooplancton échantillonné dans 38 lacs oligotrophes de la forêt boréale. Les concentrations moyennes en MeHg sont légèrement mais significativement supérieures ($P < 0{,}01$) dans le zooplancton des lacs dont le bassin versant a été récemment coupé (135 ng·g poids sec^{-1}) par rapport aux lacs dont le bassin versant a été récemment brûlé (97 ng·g poids sec^{-1}) ou n'a pas subi de perturbation (112 ng·g poids sec^{-1}). Les teneurs en MeHg dans le zooplancton provenant des lacs brûlés ne diffèrent pas significativement de celles des lacs de référence. La régression multiple pour l'ensemble des lacs montre que quatre variables indépendantes (le carbone organique dissous le pH, l'oxygène dissous dans l'eau et la biomasse des formes jeunes du zooplancton) expliquent 43% de la variation du MeHg. Le pourcentage de la variance expliqué par les régressions calculées séparément pour les trois groupes de lacs est inférieur (lacs de référence) ou supérieur (brûlés, coupés) à celui expliqué par le modèle général incluant tous les lacs. Le carbone organique dissous est la seule variable commune à tous les modèles, ce qui indique l'importance du bassin versant dans le transfert du Hg associé à la matière organique vers les lacs. Cependant, des variables influençant le taux de méthylation, telles que le pH, la concentration de sulfate et l'oxygène dissous moyen dans l'eau, expliquent aussi une partie de la variation des concentrations de MeHg du zooplancton.

Introduction

Mercury (Hg) contamination of fish is a widespread problem in remote lakes and reservoirs of the boreal forest. Volatile forms of Hg reach these regions through long-range atmospheric transport (Rudd 1995) and, after being oxidized to water-soluble forms, deposit in lakes or watersheds. In the latter, most Hg accumulates in the upper layer of soil, where it associates with the organic matter (Meili 1991). Significant disturbances in watersheds may therefore influence the transport of Hg to aquatic ecosystems. Wildfire and clear-cutting are two major perturbations affecting the boreal forest. After clear-cutting or low- to medium-intensity fires, the upper organic layer of the soil becomes more exposed to erosion, and the transfer of particulate and dissolved organic carbon (DOC) to aquatic systems may increase (Hobbie and Likens 1973). Since DOC in forest soil is an important vector of some metals (Shafer et al. 1997), the transfer of these metals, such as Hg, to streams and lakes would also increase. During high-intensity fires, on the contrary, most of the organic matter in the forest floor is mineralized (Bormann and Likens 1979) and some elements associated with it, including Hg, may be lost by volatilization.

Although numerous studies have addressed the effects of clear-cutting and fire on the mobilization of nutrients and major ions in forested soils, limited information is available on the transfer of Hg to streams and lakes as a consequence of forest disruption. In this study, we examine the relationships between fire and logging and methyl mercury (MeHg) contamination of the zooplankton community. Because DOC transfer from logged watersheds to lake water is greater than from burned or undisturbed ones, we hypothesize that zooplankton from lakes with logged watershed accumulates more MeHg than zooplankton from lakes with highly burned or undisturbed catchments. We focus on the methylated form

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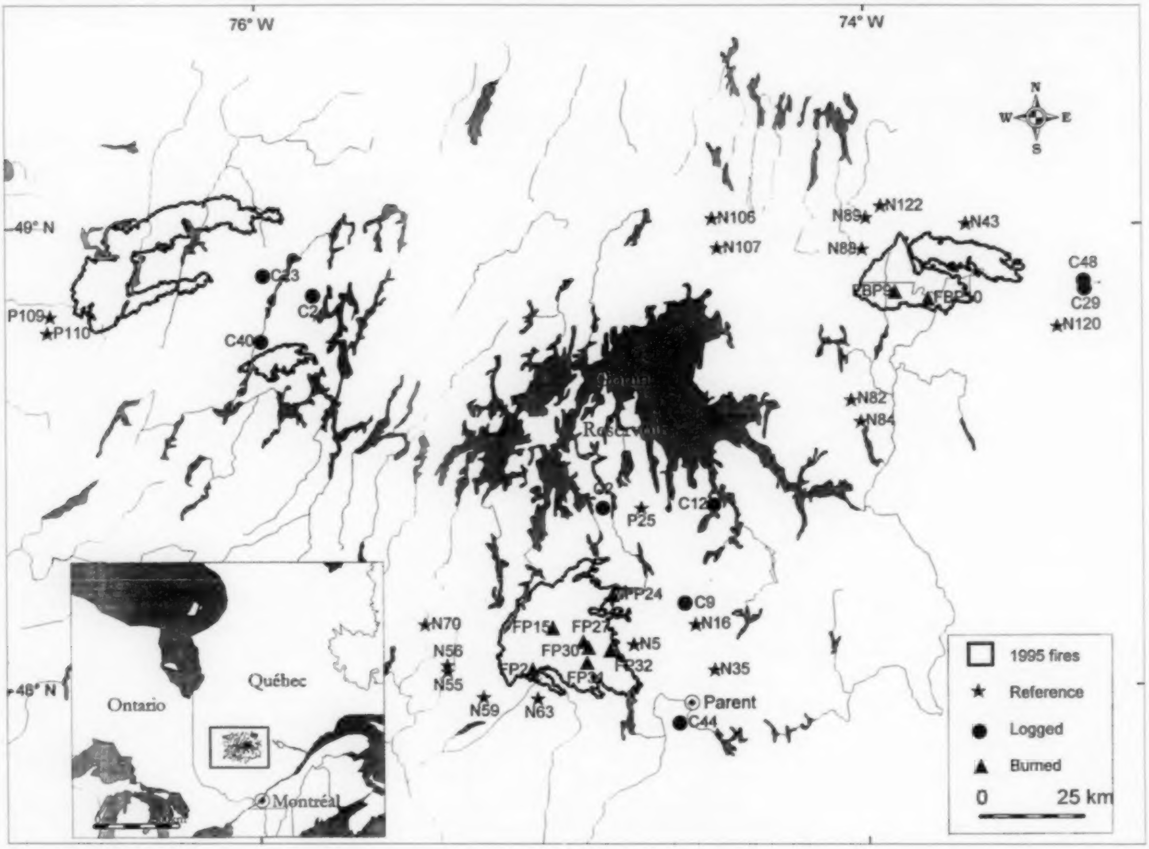
Fig. 1. Location of the study lakes.



of Hg because it can be accumulated and bioamplified along the food web. Zooplankton was selected as an indicator of MeHg contamination because these short-lived organisms can readily assimilate bioavailable MeHg from the water column and are expected to respond rapidly to changes in environmental conditions. Furthermore, they are able to directly ingest allochthonous organic carbon exported from watersheds (Hessen et al. 1990).

Materials and methods

Study site

The study was conducted in 38 headwater lakes located in a 40 000-km^2 region of central Quebec, Canada, surrounding Gouin Reservoir (Fig. 1). All the lakes are located on the Canadian Shield, where the bedrock consists mainly of granite, gneiss, mangerite, and migmatite and is covered by thin glacial tills. Soils are mostly humo-ferric podzols (Clayton et al. 1978). The lakes overlap the transition zone of the boreal mixed and coniferous forests. Wetlands (bogs, marshes, beaver ponds) are sometimes present, but their importance does not exceed 5% of the drainage area of the study lakes. The climate is continental, with a mean annual temperature of 0°C and mean annual precipitation ranging from 900 to 1000 mm (50% as snow). Three groups of lakes were selected on the basis of the type and degree of watershed disturbance: reference or null treatment ($N = 20$), logged ($N = 9$), and burned ($N = 9$). Provincial vegetation maps were used to select reference lakes with catchments that had remained undisturbed for at least 70 years. Logged lakes had 8–96% of their watershed clear-cut. In burned lakes, 50–100% of the catchment was affected by high-intensity fires. During these fires, all vegetation less than 2.5 cm in diameter was consumed, as well as the surface humus layer, resulting in bare bedrock in some areas. All logging and fires occurred during 1995, about 1 year before sampling started. The lakes were also chosen in order to minimize differences in morphometric properties between treatments (Table 1).

Water chemistry

The lakes were sampled in June, July, and September 1996. All chemical analyses were performed on duplicate integrated samples of the euphotic zone (1% of the incident light) taken with a flexible PVC tube. The samples were kept on ice and filtered (washed Gelman Supor membranes, 0.45-µm pore size) within 6 h for DOC and nitrate ($NO_3^- + NO_2^-$) analyses. Samples (500 mL) for chlorophyll *a* measurements were filtered in subdued light on Whatman GFC filters. The filters were wrapped in aluminum foil and kept at –30°C until extraction in hot ethanol (Nusch 1980). Pigment absorbances were read before and after acidification at 665 and 750 nm. Total phosphorus (TP) was measured (10-cm cuvettes, Stainton et al. 1977) after autoclaving 50-mL aliquots with 0.5 g of potassium persulfate for 45 min at 120°C. Alkalinity (Gran titration) and pH were measured within 48 h of water collection with a double-junction electrode on air-equilibrated samples. Nitrate was measured within 48 h by automated colorimetry (Lachat method No. 10-107-04-1-B). DOC was determined using a high-temperature

Table 1. Chemical and morphometric characteristics of the study lakes and of their catchments (*N* = number of observations for the three sample periods).

	Reference				Logged				Burned			
	N	Median	Mean	SD	*N*	Median	Mean	SD	*N*	Median	Mean	SD
Lake area (km^2)	20	0.4	0.5	0.2	9	0.3	0.6	0.7	9	0.4	0.6	0.53
Drainage area (km^2)	20	1.7	2.2	1.3	9	2.6	3.4	3.0	9	3.1	4.9	5.5
Drainage ratio	20	4.5	5.1	3.0	9	6.8	6.9	3.2	9	8.7	7.1	3.1
Maximum depth (m)	20	11.5	12.4	4.0	9	13.0	13.8	6.1	9	14.0	17.1	7.8
Mean depth (m)	20	4.6	4.6	1.5	9	3.7	4.3	1.6	9	5.2	5.7	1.8
Slope (%)	20	8.0	9.2	3.1	9	7.0	8.2	2.8	9	8.1	9.7	2.9
Lake water pH	60	6.6	6.6	0.3	27	6.6	6.5	0.5	27	6.7	6.7	0.4
Alkalinity (μequiv.·L^{-1})	60	46.1	54.0	28.8	27	48.3	56.0	32.0	27	58.2	68.0	41.8
DOC (mg·L^{-1})	60	5.1	5.1	1.4	27	7.1	8.0	3.9	27	5.5	5.7	1.7
TP (μg·L^{-1})	60	6.6	7.0	2.2	27	9.2	9.8	3.5	27	10.7	10.9	4.0
Chlorophyll *a* (μg·L^{-1})	60	1.9	2.0	0.8	27	2.2	2.4	0.8	27	2.9	3.1	1.1
NO_3^--N (μg·L^{-1})	60	1.1	6.5	12.5	27	1.1	3.7	7.8	27	10.0	76.6	139.0
SO_4^{2-}-S (mg·L^{-1})	60	0.9	0.9	0.2	27	0.9	0.8	0.2	27	1.7	1.7	0.4
Dissolved oxygen (mg·L^{-1})	60	8.1	8.1	1.1	27	7.9	7.8	1.2	27	7.6	7.7	1.4

Pt-catalyzed Shimadzu TOC-5000 analyzer. Sulfate (SO_4^{2-}) was analyzed by ion chromatography (Dionex DX 500). Dissolved oxygen and temperature profiles were determined with a YSI model 58 oxygen meter, and averages of these two variables in lake water were calculated and are presented in our results.

Morphometry

Lake and watershed morphometry was measured on topographic maps (1 : 20 000) and aerial photographs (1 : 15 000) using a digitizing table. Morphometric variables included drainage area (DA), lake area (LA), lake volume (VOL), maximum and mean depth, and surface of epilimnetic sediment (SES). Recent aerial photographs were used to measure the extent of perturbation by logging or fire. Mean catchment slope was estimated from the CS2 index of D'Arcy and Carignan (1997).

Zooplankton

Zooplankton was collected near the deepest point in each lake from vertical hauls using a 1-m-diameter, 200-μm-mesh net on the same dates as the water sampling. Subsamples for MeHg analysis were transferred into polypropylene bottles, immediately frozen, and later freeze-dried. Zooplankton for taxonomic identification was narcotized with carbonated water and preserved in 4% formaldehyde. Because of the large-mesh net used, the samples consisted almost exclusively of adult specimens and the larger forms of copepodites (stages IV and V). Zooplankton (5-mL subsamples) was counted and identified to the genus level, with the exception of cyclopoids and calanoids copepods. In these two suborders, only the predator calanoid *Epischura* spp. was identified; the other individuals were only discriminated as adults and immature specimens. The predators *Chaoborus* spp. and *Leptodora* ssp. were counted in the entire sample. On the basis of feeding habits, adult individuals from the different taxa and *Chaoborus* spp. were separated into three groups: filter-feeders (most cladocerans and calanoids), omnivores (cyclopoids), and predators (*Chaoborus*, *Leptodora*, and *Epischura*). A fourth group comprised immature forms of cladocerans and copepodites. Dry weight for biomass estimation was obtained from B. Pinel-Alloul et al. (unpublished data) for all taxa except *Chaoborus* spp., the dominant predator. Because of size variation of the latter species, biomass was directly measured for each sample by drying (24 h at 50°C) the individuals and weighing them on a Cahn microbalance (±0.001 mg). Results were expressed as a percentage of total biomass.

MeHg analysis

Determination of MeHg followed aqueous-phase ethylation, Tenax trap collection, isothermal gas chromatography separation, and cold-vapor atomic fluorescence detection (CVAFS) using a modification of the method described in Bloom (1989). Subsamples (0.1 g) of finely ground bulk zooplankton were digested overnight at room temperature into 10 mL of 25% KOH in methanol. The digestates (250 μL) were transferred to a reaction vessel and diluted with 150 mL of tap water. pH was adjusted to 4.9 with an acetate buffer (2 M sodium acetate in 2 M acetic acid) and 100 μL of 1% sodium tetraethylborate was then added for ethylation. Duplicate subsamples of each digestate were analyzed and averaged. The average coefficient of variation and the detection limit (3 × SD) for a 0.1-g sample were 8% and 4.8 ng Hg·g dry weight^{-1}, respectively. Analytical blanks averaged 7.8 ng Hg·g dry weight^{-1}. Certified reference materials (DORM-1, National Research Council of Canada) were run every four or five samples, and recoveries averaged 0.692 ± 0.045 mg·kg^{-1} ($N = 25$), which corresponds to 94.7 ± 6.2% of the certified value (0.731 ± 0.060 mg·kg^{-1}). MeHg measures in zooplankton samples from one burned lake were discarded due to analytical problems.

Statistical analyses

Differences in MeHg level in zooplankton as well as environmental and biological differences among the three treatments and sampling dates were tested using repeated measures ANOVA. Pearson correlation coefficients between MeHg contents in zooplankton and environmental and biological variables were calculated separately for logged, burned and control lakes. Between-treatment differences in these correlation coefficients were tested with ANCOVA. Multiple regressions were used to model the relationships among MeHg in zooplankton and the set of variables for the three treatments and for the three sampling periods. To normalize the data and reduce heteroscedasticity, the values were log transformed where required. All analyses were performed using SAS for personal computers, version 6, from SAS Institute Inc., Cary, N.C.

Results and discussion

Environmental factors

Water chemistry differed for the three groups of lakes (Table 1), with some elements also showing a seasonal variation. As expected, the most conspicuous effect of forest logging on water quality was the increase in DOC, which was greater in logged lakes than in the other two treatments (Fig. 2A). This result cannot be attributed to differences in landscape form, such as the presence of wetlands, which are sources of DOC (Mulholland and Kuenzler 1979), because the percentage of wetlands in the study lakes did not exceed 5% of the catchment. Furthermore, the levels of DOC in lake water have been related to watershed and lake morphometry, more specifically to watershed slope, to mean depth, and to the ratio between drainage area and lake area (Rasmussen et al. 1989). Since our lakes were selected in order to minimize morphometric variations (Table 1), DOC transfer to logged lakes was not more favored by the characteristics of the watershed with respect to the other two treatments. Some significant differences in morphometry were only observed between burned and reference lakes, due to the inclusion in the burned group of one deep lake (37 m) and one lake with a large drainage area.

Concentrations of sulfate and nitrate-nitrogen were substantially higher ($P < 0.01$) in burned lakes compared with the other two treatments. Concentrations of chlorophyll *a* were significantly distributed following the pattern fire > logged > control lakes ($P < 0.01$). Except in June, levels of TP were identical in burned and cut lakes, and both showed higher concentrations than reference lakes ($P < 0.01$). Concentrations of dissolved oxygen in lake water were similar for logged, burned, and reference lakes. There were no significant differences in lake water pH, alkalinity, and temperature among the three treatments, but temperature in June was significantly lower than that in July and September ($P < 0.01$).

Taxonomic composition

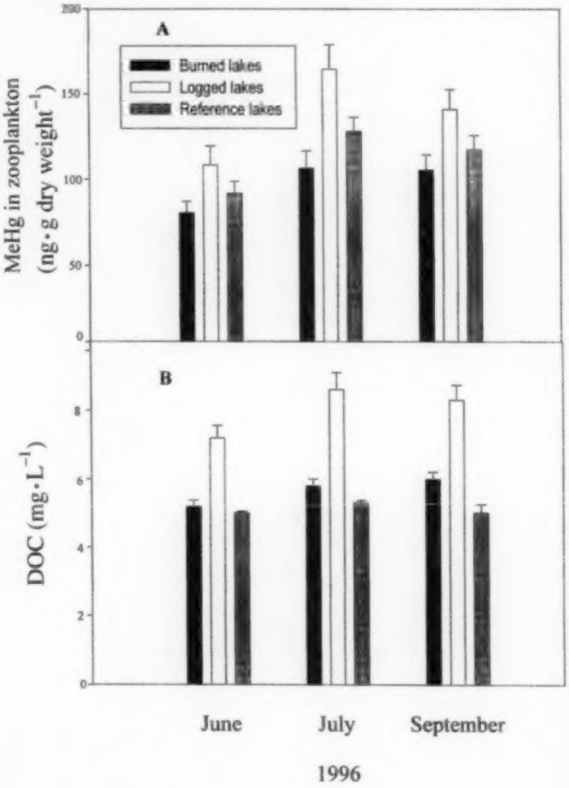
Zooplankton biomass consisted predominantly of filter-feeders (Table 2), and the relative biomass of immature zooplankton and of the three adult groups (filter-feeders, omnivores, and predators) was equivalent in the three treatments ($P > 0.10$). The biomass of immature zooplankton, however, differed for the three sampling periods ($P < 0.01$). Samples collected in June presented the greatest proportion of immature zooplankton whereas samples from July had the smallest ratio of young forms.

MeHg in zooplankton

MeHg concentrations in bulk zooplankton ranged from 35 to 377 ng·g dry weight^{-1}. This range was similar to those found by Back and Watras (1995) and Westcott and Kalff (1996) in herbivorous taxa (1–479 and 19–448 ng·g dry weight^{-1}, respectively). A narrower range (50–200 ng·g dry weight^{-1}) was reported in bulk zooplankton from undisturbed lakes of midnorthern Quebec (Plourde et al. 1997).

The three treatments showed different MeHg levels in zooplankton. In logged lakes, MeHg concentrations averaged 135 ng·g dry weight^{-1} and were significantly higher ($P < 0.01$) than those from reference and burned lakes (Fig. 2B). This result is in agreement with those of Rask et al. (1994), who found that Hg concentrations in zooplankton increased after clear-cutting of the watershed of a small humic lake. Burned lakes exhibited the lowest mean levels of MeHg (97 ng·g dry weight^{-1}) in zooplankton, but did not differ significantly from reference lakes (112 ng·g dry weight^{-1}).

Fig. 2. (A) MeHg levels in zooplankton and (B) DOC in water from logged ($N = 9$), burned ($N = 9$), and reference lakes ($N = 20$). Values are averages for each sample period. Error bars represent 1 SE.



The distribution of MeHg in zooplankton (Fig. 2B) essentially reflected that of DOC among the three groups of lakes (Fig. 2A). Thus, the highest levels of both MeHg and DOC were observed in logged lakes whereas no significant difference was found between burned and reference lakes. It is known that Hg and MeHg accumulate in the upper layer of forest floors, where they bind to humic compounds. For this reason, dissolved humic matter and particulate humic matter from exposed soils are considered as important carriers of Hg to lakes and streams (Mierle and Ingram 1991). Since DOC in lake waters essentially consists of humic substances, the similar distribution of MeHg in zooplankton and DOC among treatments during the three sampling periods suggests a relationship between the uptake of MeHg by the zooplankton and the transfer of humic matter to aquatic ecosystems, as indicated by the significant positive correlation observed between MeHg levels in zooplankton and DOC in the three groups of lakes (Table 3). Co-transport is, however, one of the mechanisms through which DOC can mediate higher biotic MeHg levels in lakes. Input of DOC may stimulate the microbial activity and the production of MeHg (McMurtry et al. 1989) and may also decrease the rates of photodegradation of MeHg in lakes (Sellers et al. 1996). In burned lakes, however, and in contrast with logged lakes, DOC loading did not increase even if soil exposure was

Table 2. Relative biomass (%) of zooplankton feeding groups by treatment and by period.

	Reference			Logged			Burned		
	June	July	Sept.	June	July	Sept.	June	July	Sept.
Filter-feeders	75.9	81.7	80.9	72.1	83.6	78.7	76.5	83.7	83.3
Omnivores	12.9	10.0	11.1	8.8	9.6	13.6	10.6	12.1	10.7
Predators	7.1	7.9	4.5	16.1	6.7	6.4	7.6	4.0	3.9
Immature individuals[a]	4.1	0.4	3.5	3.0	0.1	1.2	5.4	0.1	2.1

[a] Significant differences among periods for the three treatments considered together (ANOVA, $P < 0.05$).

Table 3. Pearson correlation coefficients observed between MeHg in zooplankton and environmental and biological characteristics of the study lakes and watersheds.

	Logged		Burned		Reference		Pooled lakes	
	N	*r*	*N*	*r*	*N*	*r*	*N*	*r*
DA/LA	9	0.54**	9	ns	20	ns	38	ns
DA/VOL	9	0.54**	9	ns	20	ns	38	0.23*
SES/VOL	9	ns	9	ns	20	ns	38	ns
Alkalinity	27	ns	24	ns	60	ns	111	–0.32***
pH	27	–0.49**	24	ns	60	ns	111	–0.42***
Temperature	27	0.43*	24	0.62**	60	ns	111	ns
Chlorophyl *a*	27	ns	24	ns	60	ns	111	ns
TP	27	ns	24	ns	60	0.27*	111	0.22*
DOC	27	0.58**	24	0.60**	60	0.32*	111	0.52***
SO_4^{-2}-S	27	0.43*	24	0.57**	60	ns	111	0.21*
NO_3^--N	27	ns	24	ns	60	ns	111	ns
Dissolved oxygen	27	–0.60***	24	–0.57**	60	–0.42***	111	–0.46***
Predators	27	ns	24	ns	60	ns	111	ns
Immature zooplankton[a]	27	ns	24	ns	60	–0.31*	111	–0.30**

Note: ns, not significant ($P > 0.05$); $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. *N* represents three sampling dates by lake. DA, drainage area; LA, lake area; SES, surface of epilimnetic sediment; VOL, volume.

[a] Biomass (relative) of immature stages of zooplankton.

accentuated after fire. Several reasons may explain why perturbation by fire did not cause an increase in MeHg concentration in zooplankton. Fire may have changed soil Hg speciation, Hg may have been lost by volatilization, and the nutrient and productivity pulse following fire may have diluted the Hg associated with the biota.

MeHg concentrations in zooplankton were also significantly correlated with other chemical characteristics of the lakes (Table 3). Some of the most significant correlations were with dissolved oxygen (–) in the three groups of lakes, temperature and sulfate (+) in logged and burned lakes, and pH (–) in logged lakes. MeHg levels in zooplankton have been reported to be correlated with some morphometric variables, such as lake area, maximum and mean depth, and the ratio between drainage area and lake volume (Westcott and Kalff 1996). In this sense, one could expect that differences in MeHg levels among logged, burned, and reference lakes would be due to variations in the morphometry rather than to differences in the treatments themselves. Among the morphometric variables, MeHg in zooplankton showed only a significant positive correlation with the ratio of the drainage area to lake volume or to lake area in logged lakes (Table 3). Both ratios were also significantly correlated with DOC (Pearson coefficient = 0.93 for ratio of drainage area to lake volume and 0.59 for ratio of drainage area to lake area). Although some authors have pointed out the epilimnetic sediment as an important site of Hg methylation (Ramlal et al. 1993), no significant relationship was observed between MeHg levels and the ratio of the surface of epilimnetic sediment to lake volume.

Because MeHg is biomagnified in food webs, one should expect to find some influence of the taxonomic composition on MeHg contamination. In our study, however, no significant correlation was found between MeHg levels in bulk zooplankton and the relative importance of each of the three groups of adult zooplankton. Because predators accounted for only a small proportion of the biomass of bulk zooplankton, samples with a greater ratio of predators were not necessarily the most contaminated with MeHg. Back and Watras (1995) also reported a lack of biomagnification of either Hg or MeHg in *Chaoborus* spp. On the other hand, MeHg levels in bulk zooplankton were inversely correlated with biomass of immature stages from reference lakes (Table 3).

Seasonal variations

Significant differences in MeHg concentrations in zooplankton were found among the three sampling dates. For the three groups of lakes, MeHg levels were significantly lower in June, then peaked in July, and decreased in September. Seasonal variation in MeHg levels in aquatic biota has also been reported by other authors (Meili and Parkman 1988; Bodaly et al. 1993). Such variations may reflect changes in the supply of dissolved and particulate material from the drainage area. Meili and Parkman (1988) observed that both total Hg in mesoplankton and DOC in lake water varied seasonally. In their work, total Hg levels in mesoplankton were

Table 4. Multiple regression models found for the three treatments and for the pooled lakes considered at the three sampling dates.

Group	Model
Logged ($N = 27$)	(1) log (MeHg) = 3.50 (±0.66) + 0.50 (±0.11) log (DOC)*** + 1.38 (±0.28) SO_4^{2-} *** – 0.12 (±0.05) DO* $R^2 = 0.77$, $SE_{est} = 0.25$, $P = 0.0000$
Burned ($N = 24$)	(2*a*) log (MeHg) = 3.52 (±0.25) + 0.62 (±0.15) log (DOC)*** $R^2 = 0.43$, $SE_{est} = 0.23$, $P = 0.0005$
	(2*b*) log (MeHg) = 4.56 (±0.40) + 0.52 (±0.15) SO_4^{2-}** – 0.11 (±0.04) chlorophyll *a** – 0.07 (±0.03) DO* $R^2 = 0.58$, $SE_{est} = 0.21$, $P = 0.0005$
Reference ($N = 60$)	(3) log (MeHg) = 4.90 (±0.42) + 0.50 (±0.16) log (DOC)** – 0.13 (±0.04) DO*** $R^2 = 0.31$, $SE_{est} = 0.30$, $P = 0.0000$
All lakes ($N = 111$)	(4) log (MeHg) = 6.59 (±0.68) + 0.32 (±0.09) log (DOC)*** – 0.10 (±0.03) DO*** – 0.25 (±0.09) pH** – 0.08 (±0.03) log (im. zoop. + 1)** $R^2 = 0.43$, $SE_{est} = 0.30$, $P = 0.0000$

Note: $*P < 0.05$;$**P < 0.01$; $***P < 0.001$. DO, dissolved oxygen; im. zoop., biomass (% relative) of immature individuals of zooplankton; SE_{est}, standard error of the estimate.

lower immediately after snowmelt and increased during the summer whereas DOC concentrations were highest in the spring. In our study, MeHg concentrations in zooplankton also were lower after snowmelt than in the subsequent periods. DOC concentrations, however, did not vary significantly from one month to another. Assuming that DOC represents an important vector of Hg for lake water (Meili 1991), these results suggest a lag period between Hg loading to the lakes from their watersheds during snowmelt and its availability to aquatic organisms as MeHg. Other studies have indicated that the rate of bacterial methylation of Hg in the sediment and in the epilimnion increases with temperature (Matilainen et al. 1991; Ramlal et al. 1993). These findings may explain why our logged and burned lakes showed a weak but significant positive correlation between MeHg in zooplankton and water temperature (Table 3).

Another explanation for the seasonal variation in MeHg levels in aquatic biota takes into account the life stage of sampled individuals. In their investigations, Parkman and Meili (1993) noted that the low Hg concentrations in zoobenthos coincided with the annual appearance of a new generation of those invertebrates. In our study, although biomass of immature zooplankton was higher in June, it represented less than 5.5% of the entire zooplankton biomass and thus is not expected to influence MeHg levels in bulk zooplankton.

Regression models

Treatment-specific multiple regressions relating MeHg levels in zooplankton to chemical, morphometric, and biological variables considered on the three sampling dates (Table 4) differed from one group of lakes to another. For logged lakes, DOC, dissolved oxygen, and sulfate explained 77% of the variance in MeHg levels in zooplankton ($SE_{est} = 0.25$) (eq. 1, Table 4). In burned lakes, DOC alone explained 43% of the variation in MeHg ($SE_{est} = 0.23$) (eq. 2*a*, Table 4). For these lakes, when DOC was excluded, the variables retained in the alternative model were sulfate, dissolved oxygen, and chlorophyll *a* ($R^2 = 0.58$, $SE_{est} = 0.21$) (eq. 2*b*, Table 4). The best regression model found for the reference lakes (eq. 3, Table 4) included DOC and dissolved oxygen and explained only 31% of the variance in MeHg ($SE_{est} = 0.29$). When all treatments were combined, DOC, pH, dissolved oxygen, and biomass of immature zooplankton were the variables retained (eq. 4, Table 4), and the model explained 43% ($SE_{est} = 0.30$) of the variance in MeHg.

DOC was the most important predictor of MeHg and, along with dissolved oxygen, was a common variable in our regressions. Other investigations have demonstrated that Hg and MeHg accumulation in zooplankton (Meili and Parkman 1988; Westcott and Kalff 1996) and in fish (McMurtry et al. 1989) increases with DOC or water color. Humic substances may, however, interfere with the availability of Hg for methylation and MeHg uptake by the biota (Grieb et al. 1990; Miskimmin et al. 1992). Thus, the relationship between MeHg bioavailability and DOC is far from being well understood.

We observed in all our models that MeHg concentration in zooplankton was inversely related to dissolved oxygen in lake water. This result is in agreement with the fact that methylation of inorganic Hg in lakes increases under low-oxygen conditions (Cossa et al. 1994). The inverse correlation between MeHg and chlorophyll *a* found in the alternative model for burned lakes may be an effect of dilution (Watras and Bloom 1994).

Even if our lakes covered a small range of pH, a negative correlation between this variable and MeHg levels in zooplankton was observed, as previously reported in other studies (Watras and Bloom 1992; Westcott and Kalff 1996). Several mechanisms have been proposed to explain why the acidity of lake water increases Hg accumulation in biota. High H^+ concentrations would affect the speciation of Hg, the rates of methylation and demethylation, as well as the physiology of the organisms (Winfrey and Rudd 1990). Laboratory studies have shown that under circumneutral conditions, a slight reduction in pH increases the rate of MeHg accumulation in fish (Ponce and Bloom 1991).

MeHg in zooplankton from logged and burned lakes was significantly correlated with sulfate, and both MeHg and sulfate showed a significant inverse collinearity with latitude. Although these results suggest a possible influence of long-range atmospheric transport from industrial regions, such a correlation was not found in reference lakes.

In conclusion, our findings suggest that in logged lakes, levels of MeHg in zooplankton may be increased due to the higher loading of organic matter bound Hg from the watershed. Furthermore, higher levels of DOC in these lakes would influence mechanisms associated with microbial methylation and with light-mediated demethylation. In comparison, intense forest fires, which also constitute a major perturbation in the boreal forest, do not appear to be linked to a comparable increase in MeHg in zooplankton.

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